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DEPARTMENT OF
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SIXTEENTH REPORT

of the

DIRECTOR OF VETERINARY SERVICES AND ANIMAL INDUSTRY

ONDERSTEPOORT,

PRETORIA.

AUGUST, 1930.

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August, 1930.

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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1906–7.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1907–8.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1908–9.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1909–10.*
First Report of the Director of Veterinary Research, August, 1911.*
Second Report of the Director of Veterinary Research, October, 1912.*
Third and Fourth Reports of the Director of Veterinary Research, November, 1915.*
Fifth and Sixth Reports of the Director of Veterinary Research, April, 1918.
Seventh and Eighth Reports of the Director of Veterinary Research, April, 1918.
Ninth and Tenth Reports of the Director of Veterinary Education and Research, April, 1923.
Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part I, September, 1926.
Eleventh and Twelfth Reports of the Director of Veterinary Education and Research, Part II, January, 1927.
Thirteenth and Fourteenth Reports of the Director of Veterinary Education and Research, Parts I and II, October, 1928.
Fifteenth Report of the Director of Veterinary Services, October, 1929.
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P. J. du Toit,
Director of Veterinary Services.

* Now out of print.

NOTE.

On the 1st April, 1929, the Division of Animal Husbandry was amalgamated with the Division of Veterinary Services and the combined Division now bears the name of DIVISION OF VETERINARY SERVICES AND ANIMAL INDUSTRY. Articles dealing with Animal Industry will, therefore, in future, be included in these Reports.

Otherwise the character of the Reports will not be altered. But an attempt will be made to publish the volumes at half-yearly intervals.

P. J. DU TOIT,
Director of Veterinary Services and Animal Industry.

Onderstepoort,
August, 1930.

Section I.

Protozoal Diseases.

G. de Kock	A Short Note on Chronic Anaplasmosis and Gonderiosis in Small Ruminants after Splenectomy.
B. S. Parkin and H. E. Hornby	A Contribution to the Study of "Immunity" in Bovine Trypanosomiasis.
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E. M. Robinson..	A Note on the Serological Diagnosis of *Trypanosoma congolense* Infection.
W. O. Neitz and A. S. Canham	A Short Note on the Spirochaetal Wound Infection of Pigs.

A Short Note on Chronic Anaplasmosis and Gonderiosis in Small Ruminants after Splenectomy.

By G. DE KOCK, M.R.C.V.S., Dr.Med.Vet., D.Sc., Sub-Director of Veterinary Services.

The object of this short paper is to place on record the occurrence of Anaplasmosis and Gonderiosis in small ruminants for several years after splenectomy. Previous observations on these cases are fully referred to in the paper of de Kock and Quinlan (1927), in which they maintain that the reactions of Anaplasmosis in splenectomised sheep are protracted over long periods with prominent and well marked remissions occurring from time to time. With regard to *Gonderia ovis*, de Kock and Quinlan (1927) are of the opinion that these parasites in South African sheep, and even in splenectomised sheep, appear to be harmless. These parasites were found to be very rare in the blood of sheep and appeared in the circulation of the splenectomised animals for many months after the operation. In sheep Nos. 8427 and 8428 they were observed from time to time for more than eighteen months. The parasites produced no changes in the blood, nor did the sheep show any visible symptoms, which could be attributed to the presence of *Gonderia ovis* in the blood.

In the following tables a record is given of the observations made on the blood of a number of animals, some of which have been kept under observation since 1924:—

Abbreviations:

S. = Slight.
A. = Anisocytosis.
Poik. = Poikilocytosis.
P. = Polychromasia.
J.B. = Jolly bodies.
P.D. = Punctate degeneration.
N. = Normoblasts.
R.C. = Red count.
R.P. = Red precipitate.
W.C. = White count.
L. = Lymphocytes.
M. = Monocytes.
N. = Neutrophiles.
E. = Eosinophiles.
B. = Basophiles or Basophilia.

SHEEP No. 8428.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
19/3/24	Intrajugularly 10 c.c. blood of sheep No. 7443.								
20/3/24	11	50	13,900	35	2	63	—	—	No changes in the blood.
2/4/24	—	—	—	—	—	—	—	—	Anaplasma rare.
9/4/24	4·6	24	11,900	53	3	43	—	1	Anaplasma; A, S.P.
9/4/24 to 1/7/24	See pages 471—11th and 12th Reports of the Dir. of Vet. Ed. & Res.								Reaction of Anaplasmosis.
3/7/24	Splenectomized.								
16/7/24	7·3	30	19,200	49	7	44	—	—	Anaplasma frequent; S.A. Gonderia.
16/7/24 to 23/4/25	See pages 472–473—11th and 12th Reports of the Dir. of Vet. Ed. & Res.								Reactions of Anaplasmosis with well marked remissions of anaemia from time to time.
27/5/25	7·2	27	16,300	66	3	30	1	—	Anaplasma and Gonderia rare, S.A.
11/8/25	7·9	30	12,400	53	3	37	5	2	Anaplasma and Gonderia.
21/9/25	7·6	30	23,300	49	4	43	3	1	Anaplasma and Gonderia.
25/11/25	7·3	29	13,800	59	4	32	3	2	Anaplasma and Gonderia rare, S.A.
17/12/25	8	25	17,500	71	2	21	5	1	Anaplasma not infrequent, S.A.
5/1/26	7·2	26	17,600	76	3	21	—	—	Anaplasma not infrequent. Gonderia rare.
22/1/26	5·5	24	12,200	69	3	27	1	—	Few Anaplasma and Gonderia.
12/2/26	6·1	24	15,000	56	5	26	3	1	S.A., S.P.
28/5/26	—	21	—	62	3	30	3	2	Anaplasma not infrequent, few Gonderia, S.A.
16/6/26	5·6	25	22,600	57	7	32	4	—	Anaplasma and Gonderia rare, S.A.
12/7/28	8·5	32	23,500	84	1	14	1	—	Gonderia rare, *no* Anaplasma seen.
19/7/28	8·3	31	28,900	58	7	32	3	—	Gonderia rare, *no* Anaplasma seen.
19/7/28	—	—	—	—	—	—	—	—	Animal destroyed in a clinically healthy state for collection of material. Study of the blood from Vena cava and Aorta revealed similar changes to those seen in the blood from the jugular.

SHEEP No. 8429

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
19/3/24	Intrajugularly 10 c.c. blood, sheep No. 7369.								
20/3/24	10	45	7,500	43	7	50	—	—	
2/4/24	—	—	—	—	—	—	—	—	S.A., Anaplasma rare.
14/4/24	3·9	17	8,100	59	9	29	3	—	Anaplasma more frequent, P.
14/4/24 to 22/9/24	See page 474, 11th and 12th Reports of the Dir. of Vet. Ed. and Res.								Reactions of Anaplasmosis.
23/9/24	Splenectomized.								
24/9/24	11	41	13,000	15	—	85	—	—	Anaplasma rare.
24/9/24 to 23/4/25	See page 475, 11th and 12th Reports of the Dir. of Vet. Ed. and Res.								Reactions of Anaplasmosis with well marked remissions of anaemia from time to time.
18/5/25	—	—	—	—	—	—	—	—	Anaplasma and Gonderia very rare, S.A.
27/5/25	6·9	30	10,000	46	5	35	13	1	Anaplasma rare, S.A.
29/5/25 to 1/8/25	—	—	—	—	—	—	—	—	Anaplasma.
11/8/25	7·9	30	19,000	30	3	54	12	1	Anaplasma not infrequent.
21/9/25	5·7	18	25,200	43	10	42	5	—	Anaplasma frequent, monocytosis with erythrophagocytosis.
24/11/25	3·2	19	20,000	40	3	53	4	—	Anaplasma not infrequent, A., P.D., J.B.
17/12/25	4·6	21	14,600	59	7	17	7	—	Anaplasma frequent, A., P.
5/1/26	7·2	26	17,600	76	3	21	—	—	Anaplasma not infrequent.
22/1/26	7·6	26	13,200	57	6	32	5	1	Anaplasma not infrequent.
12/2/26	7·9	27	15,300	63	4	28	4	1	Anaplasma not infrequent.
25/5/26	—	24	—	32	4	54	10	—	Anaplasma frequent.
11/2/27	—	—	—	—	—	—	—	—	Anaplasma not infrequent, A., J.B., P.
13/8/28	3·6	10	35,000	71	15	11	3	1	Anaplasma frequent, slight blood changes.
15/8/28	3·0	10	46,000	54	26	18	2	—	Anaplasma not infrequent, A., P., P.D.
17/8/29	2·7	9	24,000	70	13	15	1	1	Anaplasma rare, A., P., P.D., N.

SHEEP No. 8429 (*contd.*).

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
22/8/28	4·8	24	12,000	—	—	—	—	—	Few Anaplasma, S.A.
28/8/28	7·0	30	18,000	67	5	24	3	1	Few Anasplasma, S.A.
6/9/28	8·2	29	27,000	34	8	35	—	23	Anaplasma not infrequent, A., S., Poik.
26/9/28	6·9	29	12,000	—	—	—	—	—	Few Anaplasma, A., JB.,
18/10/28	5·8	25	19,400	—	—	—	—	—	Few Anaplasma, S.A., S., Poik.
1/11/28	2·5	15	24,000	70	15	14	1	—	Anaplasma rare, S.A., P., P.D., J.B.
12/11/28	7·0	29	17,000	—	—	—	—	—	Anaplasma not frequent, S.A.
19/11/28	2·8	14	30,900	48	24	27	1	—	Anaplasma not infrequent, P., Reticulocytes, N., J.B.
23/11/28	3·5	21	13,200	64	6	30	—	—	Anaplasma, A., P., P.D., N., J.B.
28/11/28	4·7	24	13,500	—	—	—	—	—	Anaplasma, S.A., J.B.
5/11/28	6·8	29	18,500	54	5	34	7	—	Anaplasma rare, S.A.
12/12/28	2·8	15	32,000	58	14	28	—	—	Anaplasma not frequent, A., S.P., N., J.B.
9/1/29	7·4	29	22,600	56	—	44	—	—	Anaplasma rare.
7/2/29	5·6	25	12,700	64	3	31	1	1	Anaplasma not infrequent, S.A.
14/2/29	5·9	19	20,000	54	6	36	4	—	Anaplasma not infrequent.
28/2/29	7·8	25	18,400	71	—	27	3	2	Anaplasma rare.
12/3/29	6·3	26	15,500	62	1	27	10	—	Anaplasma not infrequent, few N., S. P.D.
12/4/29	5·8	24	13,800	65	4	25	5	1	Anaplasma, S.A.
11/6/29	6·7	20	13,800	—	—	—	—	—	Anaplasma not infrequent., S.A.
18/6/29	5·6	22	19,400	—	—	—	—	—	Anaplasma not infrequent, P.D.
25/6/29	7·7	20	18,600	—	—	—	—	—	Anaplasma not infrequent, P.D.
2/7/29	5·5	21	24,900	50	14	30	6	—	Anaplasma frequent, S.P.

SHEEP No. 10743.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
12/3/25	6·5	24	5,900	Splenectomized.					
1/4/25	5·5	20	10,000	—	—	—	—	—	Few Anaplasma.
6/4/25	6·6	25	7,500	—	—	—	—	—	Anaplasma rare.
8/4/25	6·7	25	4,500	—	—	—	—	—	Anaplasma not infrequent, S.A., S.P.
16/4/25	7·2	25	4,700	—	—	—	—	—	Anaplasma not infrequent.
27/4/25	5·7	25	3,800	—	—	—	—	—	Anaplasma rare.
2/6/25	7·2	30	5,200	—	—	—	—	—	Anaplasma rare, S.A.
22/9/25	7·4	29	8,000	—	—	—	—	—	Anaplasma not infrequent, S.A.
22/5/26	9·3	30	6,800	58	4	36	2	—	Anaplasma rare.
27/5/26	9·0	30	8,600	55	3	40	2	—	Few Anaplasma.
13/8/28	9·1	30	10,900	—	—	—	—	—	—
11/6/29	8·7	26	8,200	—	—	—	—	—	Few Anaplasma.
18/6/29	7·9	25	8,100	—	—	—	—	—	Anaplasma rare.
25/6/29	7·9	24	7,800	—	—	—	—	—	Anaplasma rare.
2/7/29	8·5	26	8,300	—	—	—	—	—	Anaplasma rare.

SHEEP No. 16023.

Date.	R.P.	R.C.	W.C.	L.	M.	N.	E.	B.	Remarks.
11/4/27	9·0	27	5,700	—	—	—	—	—	No changes.
29/4/27	Intrajugularly 5 c.c. blood Sheep No.				8428.				
12/5/27	7·1	23	10,800	—	—	—	—	—	Few Anaplasma, S.A.
27/5/27	—	10	—	—	—	—	—	—	—
6/6/27	—	21	—	—	—	—	—	—	Anaplasma not infrequent, S.A.
29/6/27	—	14	—	—	—	—	—	—	—
1/7/27	—	15	—	—	—	—	—	—	Anaplasma not infrequent, A., P., B.
15/7/27	—	22	—	—	—	—	—	—	Anaplasma not infrequent, S.A.
18/7/27	—	17	—	—	—	—	—	—	Anaplasma not infrequent, S.A.
11/6/29	—	25	—	—	—	—	—	—	—
18/6/29	7·4	26	—	—	—	—	—	—	Anaplasma rare.
25/6/29	—	26	—	—	—	—	—	—	Anaplasma rare.
2/7/29	7·8	25	9,400	—	—	—	—	—	Anaplasma rare.

GOAT No. 8280.

Unfortunately no blood counts were made from this animal when the experiment was commenced. The number of red cells per c. mm. in goats kept under Laboratory conditions varies from 16 to 21 millions per cubic mm.

Date.	R.C.	R.P.	W.C.	L.	M.	N.	E.	B.	Remarks.
30/5/24	Splenec	tomized–	–no change	s shown.					
30/9/24	Inject i	ntraj. 20	c.c. blood	8430.					
2/12/24	—	—	—	—	—	—	—	—	Anaplasma rare.
15/12/24	—	—	—	—	—	—	—	—	Anaplasma rare.
7/1/25	—	—	—	—	—	—	—	—	Anaplasma rare.
27/2/25	—	—	—	—	—	—	—	—	Anaplasma rare.
No	further o	bservatio	ns made on	the bloo	d until t	he goat s	howed cl	inical ma	nifestations of anaemia on the
11/6/29	—	22	—	—	—	—	—	—	Anaplasma rare, S.A., S.P., S.B.
18/6/29	9·6	24	—	—	—	—	—	—	Anaplasma rare, S.A., S.P., S.B.
25/6/29	7·3	28	—	—	—	—	—	—	Anaplasma rare, S.A., S.P., S.B.
2/7/29	6·9	21	—	—	—	—	—	—	Anaplasma rare, A., S.B.

CONCLUSIONS.

From the above tables the following conclusions can be drawn :—

(1) In a large percentage of the animals the course of Anaplasmosis was of a long, protracted, chronic nature.

(2) In one sheep and one goat, Anaplasmosis has been observed for five years. The disease is still present in these animals, and they will be kept alive as long as possible, to see whether the infection will ultimately die out in a splenectomized animal.

(3) Some of these animals revealed symptoms of anaemia both clinically and haematologically. In the former there was loss of condition and marked pallor of the visible mucous membranes. Haematologically the oligocythaemia occurred in remissions. In sheep No. 8429 with a normal count of about 10 millions on the 20.3.24, 3.9 millions were registered on the 14.4.24, 3.2 millions on the 24.11.25, 3 millions on the 15.8.28, and 2.8 millions on the 19.11.28. The maximum on the other hand never exceeded 8 millions, i.e. this sheep did not recover its former normal blood count. During the very low counts the blood revealed distinct morphological changes, e.g., anisocytosis, polychromasia, punctate degeneration, normoblasts, Jolly bodies, etc.

(4) The leucocytes did not reveal marked characteristic changes, except when the blood counts registered were very low, then a monocytosis with erythrophagocytosis was manifested. After some of the remissions of severe anaemia an eosinophilia was observed.

(5) It is interesting to note that in goat No. 8280 Anaplasmosis was ultimately associated with a definite oligocythaemia. De Kock and Quinlan's earlier observations on this goat and others led them to believe that in splenectomized goats *Anaplasma* appeared in the blood, practically without signs of an oligocythaemia.

(6) It would appear that this protracted course of Anaplasmosis and Gonderiosis is probably due to the fact that the immunization mechanism has in some way been interfered with by the removal of the spleen (see also de Kock, 1929).

(7) In sheep 8428 splenectomized on the 3.7.24 *Gonderia* was still present in the blood when the animal was destroyed on the 19.7.28.

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A Contribution to the Study of "Immunity" in Bovine Trypanosomiasis.

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INTRODUCTION.

A Pan-African Veterinary Conference was held in Pretoria during August, 1929. The official report of its proceedings has not yet appeared, but we noted at the time that its Sub-Committee on Trypanosomiasis did not hold an optimistic view of the part that active immunization or the attempts to breed immune races of domestic animals would take in combating these diseases. It was stated, for example, that " the existence of small herds in tsetse-fly belts is reported from several countries, and at one time hopes were high that selective breeding would produce races of domestic animals possessing an immunity-tolerance towards all species of trypanosomes similar to that possessed by game. It would seem, however, that the ability of these herds to live in their particular fly-belts, is due to a limited immunity-tolerance towards local strains of trypanosomes, and that even this is easily broken down by disease, e.g. rinderpest, and other hardships."

The high hopes referred to were expressed by Roubaud (1922) among others when, after describing some West African races of cattle which appeared to be immune against trypanosomiasis, he stated " it is on the study and careful selection and distribution of these small resistant races that the future of the cattle-raising industry in tropical Africa depends." More recently Buchanan Smith (1928) stated that: " it is desirable and almost imperative further to explore the possibilities of the genetical line of attack."

Although Laveran (1914) claimed that goats which had recovered from a trypanosome infection did not transmit any appreciable immunity to their offspring yet, as Brumpt pointed out at the time there must be some measure of inherited resistance or natural selection could not have evolved immune races of domestic animals. In 1926, one of us examined a small herd of eight cattle living in apparently good health, in a thick fly-belt near Tabora. These cattle were the progeny of three cows and were the only survivors and descendants of approximately a hundred head brought there between 1913 and 1920. This observation reconciles the view of Brumpt that there is some inherited immunity with that of Laveran that hereditary transmission of immunity is feeble or rare. It is certain that it is only

with the greatest difficulty and at great cost of lives that resistant races can be built up by natural selection. So far no great success has attended the few attempts made to bring this about by artificial selection.

That this matter of fostering and improving local races of naturally immune cattle, has become more prominent during the last decade, may be due to the lack of success which has attended all efforts to provide a practical method of artificially immunizing domestic animals against trypanosomiasis. Much attention has naturally been paid to immunization in the past, because of the immediate and valuable uses this could be put to by those living in or near fly-belts. What, however, has often been overlooked, in this connection, is the possibility that such a discovery might complicate rather than solve the main problems connected with trypanosomiasis. The type of immunity likely to result would probably be of the same nature as that exhibited by game and by local naturally resistant races of domestic animals, namely an immunity-tolerance with the survival of the parasite in the host. The ultimate aim of those who combat disease should be its complete eradication. Consequently, the production of large numbers of carriers of trypanosomes would be detrimental to the attainment of this goal. It was partly on this account, we think, that the Pan-African Veterinary Conference did not enthusiastically commend endeavours to confer immunity. Nevertheless expediency demands these endeavours, and they will doubtless continue to be made in the future to enable the occupation of tsetse-fly areas to be proceeded with until such time that the eradication of fly becomes a practical proposition.

At first the attempts to confer immunity followed, in general, the lines which had been found successful in bacteriology. The experiments have been carried out chiefly on small laboratory animals. As none of these early endeavours shewed sufficient promise to justify their application under field conditions, it is unnecessary to deal with them further. There, however, has been in recent years a distinct tendency to concentrate on the production of animals which, notwithstanding the fact that they harbour the parasites, yet remain in apparently good health.

Even in the early days of drug treatment of trypanosomiasis it was observed that many of the " recovered " animals continued to harbour parasites. These, however, under such conditions were found only in comparatively small numbers and the animal's health was not appreciably affected. This is a state which resembles that found in game living in the tsetse-fly belts. Duke (1912) records the recovery of *T. uniforme* from clinically healthy antelopes ten months after they were last exposed to infection.

Bevan (1928) has recently endeavoured to bring about a similar state in bovines. His method consists of the treatment with medicinal doses of Antimony Potassium Tartrate of bovines infected with *T. congolense*. In all he gives three doses, the time of dosing being determined by the time of re-appearance of the trypanosomes in the blood. He lays particular stress on this " timing " of the injection. Thereafter he finds that the animals recover and resist artificial reinoculation with trypanosomes of the same species. These animals,

he hopes, would be resistant to natural infection when exposed in a fly-belt.

Our intention in this paper is to record some observations which, while exposing the weak points of the existing schemes of immunization, may yet contribute towards their ultimate successful development. These observations were made during experiments planned for a different purpose.

IMMUNITY AND PREMUNITION.

It is considered advisable to adopt the suggestion of some French workers and restrict the use of the word " immunity " to the denotation of a condition of resistance to infection which is accompanied by total inability of the invading parasite to survive in the body of the immune animal and " premunition " to the condition of resistance which is accompanied by, or even dependent on, the survival of the parasite in the host. The latter is the state found in antelopes living in a fly-belt which, notwithstanding the fact that the parasites can multiply, to a certain extent, in their blood, do not suffer appreciably from the infection. Of a similar nature is the " immunity " in many other protozoan diseases.

RESTRICTION OF SUBJECT.

Cattle in Africa, although susceptible to a number of different species of trypanosomes, rarely suffer, to any great extent, from the effects of trypanosomes, other than *T. congolense* and *T. vivax*. All efforts, therefore, to produce resistance should be directed against these two species. As far as our present knowledge goes, it does not appear to be possible to obtain a true immunity against these trypanosomes by artificial means. It yet remains to be proved that the resistance in nature is other than premunition. Consequently, it is considered that the only form of enduring resistance which one can, at present, attempt to confer, is that of premunition. Furthermore, as from experience, it is known that *T. vivax* disease is less severe and more easily treated than *T. congolense* infection, the evolvement of a practical method of premunition against *T. congolense* would likely solve the problem also in connection with *T. vivax* infection. Our remarks from now on are restricted to those relevant to the subject of premunizing of cattle against *T. congolense*.

COURSE OF THE UNTREATED DISEASE.

The inoculation of a susceptible bovine with an infective dose of *T. congolense* is usually followed by a period of from one to three weeks before the appearance of trypanosomes in the blood stream and the commencement of the thermal reaction. In our experiments we generally used, as an inoculum 5 c.c. of citrated guinea-pig blood (equal parts of 1 per cent. citrate solution and blood rich in trypanosomes). We, however, found that in most cases this injection, given subcutaneously, was followed by as short an incubation period as one of a week or less.

In the field one meets with cases of acute, sub-acute, and chronic infections. Our animals were kept under very good conditions as regards stabling, food and general attention. Under such conditions

the untreated disease almost invariably ran a chronic course, marked by an irregular low fever and by loss of condition. Mere superficial examination of an infected animal would reveal, as only signs of illness, an unthrifty appearance, an abnormally frequent pulse and an irregular hyperthermia. Blood-smear examination might show the trypanosomes, but not those changes in the red cells (anisocytosis, polychromasia, etc.), which are generally associated with profound anaemia. It was found that one of the best methods for determining the degree of advancement of the disease was by means of a red cell count. *T. congolense* infection, apart from the thermal reaction, appears to be an almost pure anaemia, such as results from mechanical abstraction of blood. The regeneration mechanism of the blood does not appear to function with any great efficiency.

The type of bovine used in our experiments has a red cell count of eight to ten millions per c.mm. If such an animal is well fed and well cared for, it can lose about half of these without any appreciable loss of condition. If, however, the figure falls below this level of safety, then no care can prevent it losing condition. The undernourishment shows up conspicuously in connection with the skin which becomes dry and scurfy. The pulse, on account of loss of haemoglobin which progresses in a manner parallel to that of the red cells, becomes more frequent. The visible mucosae, however, do not show changes commensurate with the anaemia present. Below we give a chart of bovine 2714, a typical untreated case of *T. congolense* infection, which shows (1) the temperature curve, (2) the red cell count and (3) the occurrence of trypanosomes from the time of inoculation to the definite assumption of the chronic state.

The ultimate fate of such an animal as bovine 2714 depends a good deal on the care and attention it receives. Once the red cell count falls below what we regard as the level of safety, i.e. five million per c.mm., the animal becomes incapable of thriving, loses condition, and is unable to work. It also necessarily loses resistance to intercurrent infections. Consequently, under the hardships which are normal for most African cattle, chronic trypanosomiasis usually terminates fatally. There is no doubt in our minds that even our well attended experimental animals would die if left untreated, though actually only one of our controls did die during several months of observation. This was chiefly due to the fact that bovines when in the last stages of the disease were submitted to one or other of the methods of treatment being experimented with.

For the purpose of our experiments, we regarded an animal as being affected with the chronic infection, to distinguish it from the premunized animal, when there was a more or less rapid continuous loss of condition associated with a marked ultimate decrease in the red cell count.

PRODUCTION OF PREMUNITION.

Under favourable conditions the majority of cattle show a considerable natural resistance to *T. congolense* infection which, in such circumstances, usually takes a chronic course. If a trypanocidal drug is given to an infected animal, especially to one which has already reached the chronic stage, we found that this administration was in

most cases sufficient to turn the state of the animal's condition from one of a chronic nature to one of premunition. We have mentioned above Bevan's method of attaining this object. In our experiments we found that it was not necessary to "time" the injections, we could obtain premunition without any particular consideration of the time of the appearance of the trypanosomes in the blood. The natural essential, of course, was that the treatment be not pushed to such an extent as to produce complete sterilization of the animal.

We may take, as an example of the production of premunition, bovine 2709. At the time of infection its red cell count was 8 millions per c.mm. Five weeks later its count had fallen to $3\frac{1}{2}$ millions. It was then treated at four-day intervals with small doses of a trypanocidal drug which, as our previous experience had indicated, would not be sufficient to sterilize it. Thereafter the animal made a very slow, almost imperceptible, improvement, and by the end of seven months, although trypanosomes were occasionally found, it attained once more a red cell count of 8 millions per c.m.. Its condition at this time was very good.

An example of the production of premunition by a different method is furnished by bovine 2714, the history of whose early infection we have already considered. This animal was a typical case of *T. congolense* infection with a red cell count in the neighbourhood of 4 millions per c.mm. Six months after infection treatment resulted in it being sterilized. A state of premunition was then brought about by re-inoculating it with the original strain. Subsequently trypanosomes were found occasionally in the blood-smears, but the animal did not develop anaemia, its red cell count going up to nearly 9 millions per c.mm.

There is no difficulty in defining the state of these two bovines as one of premunition. The trypanosomes were found occasionally in the blood smears, but notwithstanding their presence, anaemia was absent. Furthermore, their condition and appearance was good and growth was satisfactory.

There is, however, no sharp line between the state of the chronic infection and that of premunition and it is at times difficult to come to a decision whether the animal is in one or the other state. Take, for example, the two bovines 2464 and 2468. For the two months following infection the course of the disease was somewhat similar to that of those already referred to. Treatment resulted in the checking of the disease and in some improvement in condition. Trypanosomes were never absent for long. The animals remained more or less in this state for the next three months with red cell counts of 5 and $4\frac{1}{2}$ millions per c.mm. respectively. Condition was fair, growth was fairly satisfactory, they would probably be capable of light work, but there is no doubt that, under unfavourable conditions, such anaemic animals would not have been able to retain such condition as they had, and would have relapsed into a state of chronic infection.

PREMUNITION AGAINST HOMOLOGOUS AND HETEROLOGOUS STRAINS.

So far we have been dealing with infection by, and premunition against, a single strain of *T. congolense*. We must now pass to the

consideration of the extent to which the premunition against a strain is effective against a different strain. Before this was tested out, the effect of inoculating cattle with trypanosomes of the strain against which they were premunized was tried. For example, when we believed bovines 2709, 2464 and 2468 to be premunized against the strain in use which was obtained from Zululand, we inoculated them and three controls (bovines 3516, 3525 and 3627) each with 5 c.c. of citrated blood of guinea-pigs infected with the homologous strain. The results are set out in Table I, and show quite clearly the difference between the reactions in the susceptible controls and the entire lack of reaction on the part of the premunized ones. Actually these latter continued to improve in condition during the test.

After the bovines 2709, 2464 and 2468 had shown that they were entirely unaffected by further inoculations with trypanosomes of the Zululand strain, each was inoculated with 10 c.c. of citrated guinea-pig blood of a strain, called hereafter the Rhodesian Strain, which had been obtained by the Director of this Laboratory through the courtesy of Mr. Ll. E. W. Bevan, Director of Veterinary Research, Southern Rhodesia. The results are set out in Table II and show that the bovines premunized against the Zululand strain reacted to the inoculation with the Rhodesian strain in a manner very similar to the reaction obtained by inoculation of susceptible bovines with the latter strain. There was apparently no difference between the premunized animals and the susceptible controls as far as concerns the thermal reaction, the period of incubation and the multiplication of the trypanosomes in the tissues.

In association with these premunition tests was used the Adhesion Phenomenon. This is now so well known that there is no necessity for us to describe it. Those desiring further information, should consult the article by Davis and Browne (1927) or the one by Johnson and Lester (1928). The usual technique was employed and the test was carried out on the plasma or serum, no difference in the action of these being noted. Throughout the test, the bovines premunized against the Zululand strain, gave positive results with this strain. At the time of inoculation of these animals with the Rhodesian strain, they gave negative results to this strain and continued to give such results until four weeks after the inoculation when a change to positive was recorded. Negative controls in every case gave negative results.

There was, however, a slight difference in the effect of the inoculation on the red cell count. In the case of the premunized bovines with an initial low red cell count, there was an increase during the four weeks following the inoculation with the Rhodesian strain, i.e. there was a continuation of the process of recovery from the Zululand strain. In those premunized bovines with a high red cell count, the decrease was slower and not so marked as in the control. Now the presence of a large number of trypanosomes in the circulation of susceptible bovines results in a marked diminution of the red cells. The comparative absence of this reduction in the cattle premunized against the Zululand strain after inoculation with the Rhodesian strain, notwithstanding the fact that there were numerous trypanosomes present, argues the existence or rather development in these animals of an improved mechanism for dealing with the destructive effects of the trypanosomes.

We see, therefore, that a bovine may be strongly premunized against one strain of T. congolense and yet be without appreciable resistance to the invasion and multiplication of trypanosomes of other strains. This is the weak point of some of the existing schemes for production of artificial immunization. The advantage which premunition against one strain gives to a bovine infected with another strain is that the animal is not affected to the same extent with anaemia and consequently a certain amount of protection against the dangers of anaemia is afforded with the result that such an animal would have a better opportunity to attain to a state of premunition against the new strain. The tax on the blood-forming tissues would probably only become of importance when an animal lives under unfavourable conditions. Thus it is easy to understand that, although individuals of a small herd of cattle under otherwise favourable conditions can live in tsetse-fly belts by developing premunition against various strains of trypanosomes, yet, if these " immune " animals are exposed to infection by an additional strain when under unfavourable conditions, they may then be unable to withstand the new attack and their immunity would appear to break down. Realization of the true nature of this breakdown of immunity is necessary to the successful development of any scheme of immunization.

SUMMARY.

1. As bovines are chiefly affected by *T. congolense* and *T. vivax*, all efforts to immunize them should be aimed at these two species.

2. It is probable that a method of immunization against *T. congolense* would also be successful against *T. vivax*.

3. Untreated *T. congolense* infection of bovines under favourable conditions usually runs a chronic course with the appearance of unthriftiness, frequent pulse, thermal reaction and anaemia.

4. The administration of a trypanocidal drug often changes the chronic state to one of premunition. By this means it is not difficult to premunize a bovine against a single strain of *T. congolense*.

5. Bovines premunized against a particular strain of *T. congolense* are unaffected by reinoculation with the same strain.

6. Bovines premunized against one strain of *T. congolense* are practically as susceptible to inoculation with a different strain as bovines not so premunized.

7. Bovines premunized against a particular strain give a strongly positive Adhesion Reaction with the same strain but not with a different strain.

8. Bovines, premunized against a particular strain and then inoculated with a different strain, do not show, as reflected by the red cell count, such a progressive anaemia as bovines not so premunized. This point in favour of the premunized bovine might be a factor, under natural, especially favourable conditions, which might enable a greater proportion of bovines to become premunized against a second strain of *T. congolense*.

Note by H. E. Hornby.—Most of the observations which form the basis of this article were made during three months of leave from Tanganyika Territory, which I spent at Onderstepoort. The hospitality extended by this Institute to visiting men of science is traditional; nevertheless I wish to express my gratitude personally to the Director and his Staff for kindness, assistance and provision of facilities for study.

TABLE I.

T. congolense.—INOCULATION OF PREMUNIZED BOVINES WITH HOMOLOGOUS STRAIN.

Bovines.	Description of State.	Inoculum.	Presence of Trypanosomes on Days after Inoculation.													Red Cell Count.		Adhesions Phenomenon.†		
			2	4	6	8	10	12	14	16	18	20	22	24	26	Before Inoculation.	4 Weeks after Inoculation.	Before Inoculation.	2 Weeks after Inoculation.	4 Weeks after Inoculation.
B. 2709..	Z-premune*	Z-strain	—	—	—	—	—	—	—	—	—	—	—	—	—	8·7	9·2	+	+	+
B. 2464..	,,	,,	—	—	—	—	2+	3+	—	—	2+	—	1+	—	2+	—	—	+	+	+
B. 2468..	,,	,,	—	—	—	—	—	2+	—	—	1+	—	—	—	1+	—	—	+	+	+
B. 3506..	Control.....	,,	—	—	—	2+	2+	2+	2+	2+	1+	3+	1+	3+	3+	9·1	6·8	—	±	+
B. 3525..	,, ...	,,	—	—	2+	3+	1+	4+	1+	3+	1+	3+	1+	1+	1+	10·0	5·6	—	—	+
B. 3627..	,, ...	,,	—	1+	2+	4+	3+	4+	3+	2+	3+	3+	3+	1+	3+	8·8	4·3	Not tested.		

* Z.-premune—Premunised against Z or Zululand strain.

Trypanosomes absent (—), very rare (1+), rare (2+), frequent (3+), very frequent (4+).

† Adhesion Phenomenon negative (—), definite (±), complete (+).

TABLE II.

T. congolense.—INOCULATION OF PREMUNIZED BOVINES WITH HETEROLOGOUS STRAIN.

Bovines.	Description of State.	Inoculum.	Presence of Trypanosomes on Days after Inoculation.													Red Cell Count.			Adhesions Phenomenon.†				
																			To Z-strain.		To R-strain.		
			2	4	6	8	10	12	14	16	18	20	22	24	26	Before Inoculation.	Four Weeks after Inoculation.	Eight Weeks after Inoculation.	Before Inoculation.	Two Weeks after Inoculation.	Before Inoculation.	Two Weeks after Inoculation.	Four Weeks after Inoculation.
B. 2709	Z-premune*	R-strain*	—	—	1+	2+	3+	4+	1+	3+	1+	2+	2+	1+		9·8	8·2	6·3	+	+	—	—	+
B. 2714	,,	,,	—	—	1+	2+	3+	2+	2+	3+	3+	1+	2+	2+		10·1	8·8	5·1	+	+	—	±	+
B. 2464	,,	,,	1+	—	—	1+	3+	3+	1+	3+	2+	1+	1+	1+		5·2	6·1	—	+	+	—	—	+
B. 2468	,,	,,	—	2+	—	—	3+	2+	3+	1+	1+	4+	2+	1+		4·4	5·0	—	+	+	—	±	+
B. 2994	Control....	,,	—	—	—	2+	3+	4+	2+	3+	3+	1+	2+	2+		—	4·6	—	—	—	—	±	+
B. 3636	.. ..	,,	—	—	2+	2+	3+	4+	4+	2+	2+	2+	3+	3+		8·4	3·6	—	Not tested.		Not tested.		

* Z-premune—Premunised against Zululand strain.

* R-strain—Rhodesian strain.

Trypanosomes absent (—), very rare (1+), rare (2+), frequent (3+), very frequent (4+).

† Adhesions phenomenon negative (—), definite (±), complete (+).

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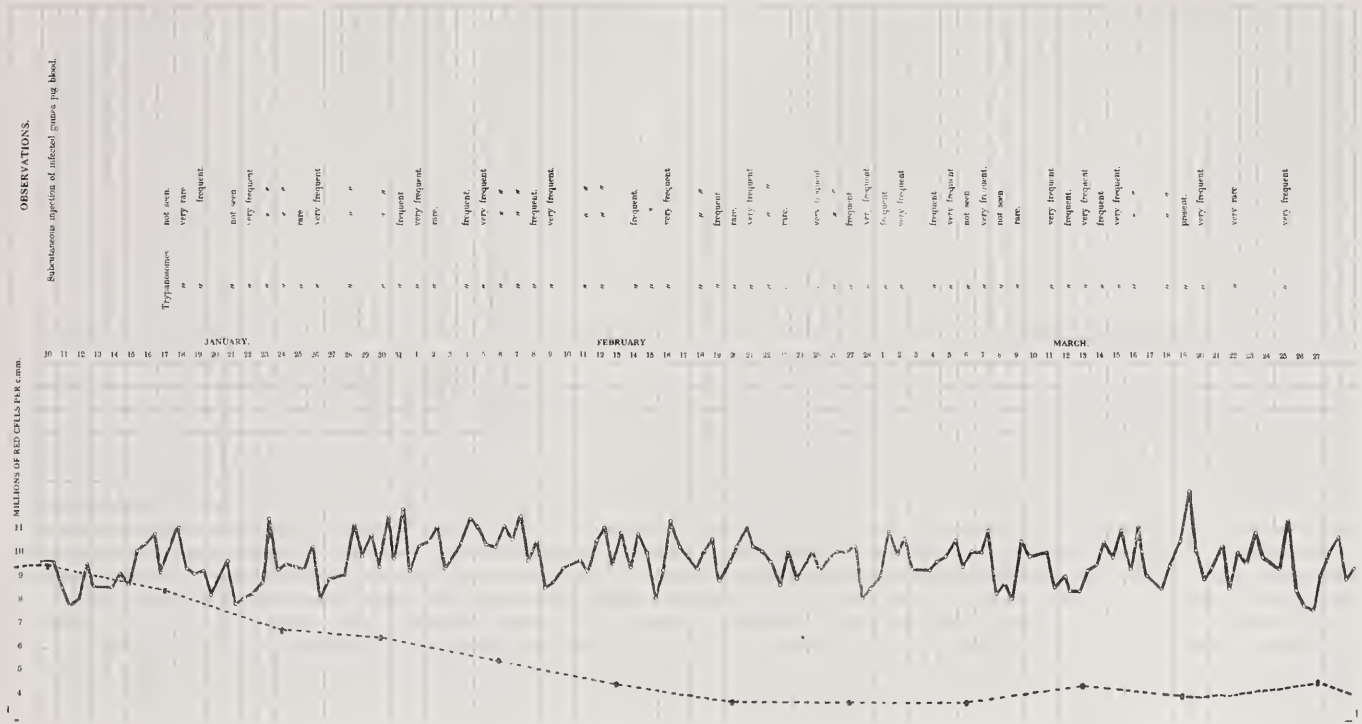
BOVINE 2714. A CHRONIC CASE OF T. CONGOLENSE INFECTION.



16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

A Study of Bovine Trypanosomiasis.

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I. INTRODUCTION.

This work was originally commenced with the object of testing out various drugs for the treatment of bovine trypanosomiasis, a serious drawback to the advancement of the cattle industry in Zululand. Considerable progress was made in the control of this disease after the introduction by Curson (1928), on a large scale, of the Potassium Antimony Tartrate treatment. The disadvantages in the use of this drug have, however, mitigated, to a considerable extent, against its wide application. Curson himself recognized the two chief disadvantages when he stated " . . . tartar emetic is admittedly far from being an ideal drug chiefly on account of its temporary beneficial action and the disadvantages resulting from faulty administration . . ." As a consequence in the forefront of these investigations were kept (*a*) ease of administration and application, (*b*) efficacy of treatment against *T. congolense* infection and to a less degree against *T. vivax* and *T. brucei* infections, and (*c*) cost of method of treatment. However, as the work progressed, it was soon realized that the scope would have to be considerably increased to determine the possibilities under laboratory and field conditions of various points which appeared as a result of the institution of the new method of chemotherapy of bovine trypanosomiasis to be hereafter described. There are consequently included chapters on premunition, identification of strains of *T. congolense*, etc. Portions of the work already performed are not sufficiently complete to justify their publication in detail; short references to these parts, however, are made.

II. DISCUSSION OF POTASSIUM ANTIMONY TARTRATE TREATMENT.

For many years now chief reliance for the treatment of bovine trypanosomiasis caused by *T. congolense* and *T. vivax* has been placed on the intravenous injection of Potassium Antimony Tartrate, The number of injections in a course of treatment, the dosage and the intervals at which the injections are given vary somewhat in the different areas. In Zululand a single injection consists of 1 to 1.5 gm. in 20 to 30 c.c. water, and, as a routine measure, five injections are given on consecutive days. The Tanganyika authorities employ eight consecutive weekly injections of 25 c.c., each of a 4 per cent. solution. Other countries utilize one or other of these two, which represent the shortest and the longest courses of treatment, or slight modifications of them.

Ease of Administration and Application.

Potassium Antimony Tartrate is usually given intrajugularly. Consequently the animal in most cases has to be controlled by casting or some other complicated method. Bovines living in or near tsetse fly areas are not usually of much value and are left to range practically uncontrolled. Any treatment instituted therefore must take into consideration that the animals are often troublesome to collect and difficult to handle and control. If the drug utilized in the treatment is to be injected, the administration by the subcutaneous or intramuscular routes instead of by the intravenous, would simplify matters considerably.

The operation of intrajugular injection is by no means a simple one. Skill is especially necessary when the material to be injected is as irritant as the solution of Potassium Antimony Tartrate is. The local effects of faulty administration might be extremely serious, interfering often with the subsequent administration of the drug. A trypanocidal drug which could be administered by the subcutaneous or intramuscular routes would be a great advance in the chemotherapy of bovine trypanosomiasis.

Efficacy of Treatment.

Potassium Antimony Tartrate has been found to be of little or no value in treatment of bovine trypanosomiasis caused by *T. brucei*. Even though this parasite is comparatively rare in bovines and does not produce marked ill-effects in the animals, yet it would certainly add to the value of any treatment if it could be shown that it was also effective against *T. brucei* type of bovine trypanosomiasis. The efficacy of Potassium Antimony Tartrate in bovine trypanosomiasis caused by *T. congolense* and *T. vivax* does not need to be commented on here as the references in literature on this point are numerous.

Cost of Treatment.

A decided advantage in favour of the use of Potassium Antimony Tartrate is its cheapness. In Africa, especially in those areas where it becomes necessary to institute treatment for trypanosomiasis, the bovines are of small value. Consequently any treatment employed must be cheap enough to justify its use on an economical basis. Potassium Antimony Tartrate fulfils this condition ideally, the cost of an average dose being approximately one-fifth of a penny. The cost of the drug is thus of practically no hindrance to its wide application in bovines of even small value. Even though the first essential in the use of a treatment for such bovines is cheapness yet it should naturally be an ideal to provide the most effective method of treatment possible and then to endeavour to cover any additional expense connected with the drug by the improvement of the type and class of animals kept. The introduction of improved breeds in any area is often dependent on the introduction of better methods of treatment of diseases; the native stock, usually more resistant, are replaced only when improved methods of disease control enable the more susceptible but greater revenue producing improved cattle to survive.

III. ANTIMOSAN THERAPY.

On account of the known efficacy of Potassium Antimony Tartrate against the most important of the trypanosomes affecting bovines namely *T. congolense*, it was decided to choose from the antimony compounds for the first investigations. This work commenced in January, 1929, and is still being continued. The drug Antimosan chosen at that time is the one still being experimented with. It has proved to give such excellent results in bovine trypanosomiasis that it was felt that a thorough investigation into its use and application was fully justified.

Antimosan is a product of the I.G. Farbenindustrie Aktiengesellschaft, and is described as a complex compound of a trivalent antimony preparation with a pyrocatechin derivative. The manufacturers state that a series of five injections of 3 gm. each at intervals of 8 days, is generally required for bringing about a cure. They give no details in connection with tests carried out. The drug is given intravenously or in case of need subcutaneously. For veterinary purposes it is put up in ampoules of 25 c.c. 12 per cent. solution, ampoules of 40 c.c. 7 per cent. solution, and a tablet form of 1 gm. each. The supplies provided originally by the manufacturers for test consisted of the potassium salt of Antimosan. More recently they have introduced the sodium salt. The antimony content of the Potassium Antimosan which was used throughout the major portion of the experiments to be described, is as follows:—

Antimosan solution 7 per cent. 1 c.c. = 0.0085 gm. Sb.
Antimosan solution 12 per cent. 1 c.c. = 0.015 gm. Sb.
Antimosan solid 12.5 per cent. = Sb.-content.
Potassium Antimony Tartrate 36.2 per cent. = Sb.-content.

The tablets differ slightly from the solid on account of it being a mixture. For purposes of comparison the antimony content of Potassium Antimony Tartrate is added. The 12 per cent. solution is hypertonic and the 7 per cent. solution is isotonic. At the time of receipt of the Antimosan the hypertonic solution was regarded as being non-irritant. It has since been shown, however, to be slightly irritant especially to horses (see article on equine trypanosomiasis in this Report). If a calculation be made on the Sb-content of Potassium Antimony Tartrate and Antimosan, a dose of 1 gm. of the former corresponds approximately to a dose of 25 c.c. of the 12 per cent. solution or 40 c.c. of a 7 per cent. solution of the latter. Consequently the majority of the experiments were carried out with these doses.

The reasons activating the selection of this drug were firstly because it was an antimony compound, and secondly because it was claimed that it could be given subcutaneously.

The literature in connection with the use of Antimosan available at the commencement of these experiments was very meagre. Curson (1926) studied its effect on *T. congolense* and *T. brucei* infections of white mice, and came to the conclusion that it was a good curative agent for *T. congolense* infection but not a promising one for *T. brucei* infection. In the Annual Report of the Tanganyika Territory

(1927) is recorded the treatment of nine bovines with 50 to 60 c.c. Antimosan 5 per cent. solution at intervals varying from 2 to 7 days, the drug being given both intravenously and subcutaneously. The results were regarded as being promising. In the Report of the same Territory (1928) of the following year, there are tabulated the results of further treatments. According to this table Antimosan was given, at the rate of approximately 0.015 gm. per Kg. up to 10 doses in some cases being given and it was also at times used in combination with Potassium Antimony Tartrate. The summary concludes with the following words:—" To-day, as a routine procedure of treatment, we favour intravenous injections of Antimosan at intervals of a week; but we hope soon to be able to give much more definite advice." The use of the intravenous method of administration, in the writer's opinion, immediately destroys one of the chief advantages of this drug. Another point of interest in the Report of 1927 (p. 31) is the statement that old-standing cases are much more responsive to the drug's action. This statement refers to the action of Antimony compounds in general. Curson (1928), however, says " In some early cases one dose (*of Potassium Antimony Tartrate*) appeared sufficient . . . And for a few animals that have survived one or more Nagana seasons as many as ten inoculations have been ineffective." This point of the relative effectiveness of early and late treatment will be dealt with towards the end of this Chapter. It may just be mentioned here that, as far as treatment of bovine trypanosomiasis due to *T. congolense* with Antimosan is concerned, the writer has come to the conclusion that late treatment is very much more efficacious.

As far as the ease of administration is concerned, Antimosan is immeasurably superior to Potassium Antimony Tartrate. There is when the subcutaneous route is employed no necessity for elaborate methods of control, there is elimination of the often severe complications associated with the escape of the irritant Potassium Antimony Tartrate solution into the perivascular tissue and further, as will be later shown, there is a great reduction of the number of administrations necessary to bring about sterilization or to maintain the bovines in health in areas where they are constantly exposed to re-infection. For the subcutaneous administration the use of a crush, such as can be easily constructed locally and such as is often required for other operations of a like nature, e.g. anthrax immunization, provides a simple and speedy method of control for the injection. The handling of the individual animals is almost entirely eliminated.

No complications or objectionable sequelae of any importance have been noted in connection with the subcutaneous administration of Antimosan in bovines. If reference be made to the article by the author in this Report (1930), it will be seen that, in horses, the 12 per cent. solution produced at times considerable damage to the tissues at the site of inoculation. In bovines, however, no damage of any moment was observed from the use of this hypertonic solution. At times swellings appeared as a result of the injection which was carried out without any special precautions, but in only one case was an abscess observed and at no time was there interference with subsequent injections. For this reason the treatments hereafter described were all carried out with the 12 per cent. solution given subcutaneously. The

advantage of the use of this solution is that there is a considerable reduction of the bulk of the solution as compared with the 7 per cent. solution, but this advantage possibly is not great enough to justify the continuation of its use. Its replacement under field conditions by the 7 per cent. solution is indicated. All our field trials are being carried out with the isotonic solution.

The cost of treatment with Antimosan at its present price, taking into consideration the value of individual bovines likely to be infected with trypanosomiasis, is a factor which may interfere considerably with the wide use which is justified by its efficacy. The cost of the treatment which is now recommended for the purpose of maintaining a bovine in health when exposed, more or less constantly, to infection will be about 10s. per bovine per annum. On the face of it, this expenditure does not perhaps appear to be of much importance, but it is hardly justified on an economical basis. The hope is that improvement in the stock which might be expected to follow on the introduction of improved chemotherapy may justify such an expenditure.

As bovine trypanosomiasis may be the result of infection with *T. congolense*, *T. vivax* and/or *T. brucei*, it becomes necessary to examine the efficacy of Antimosan against all these species of trypanosomes. Naturally particular importance would be attached to its efficacy against *T. congolense*. The chemotherapy of these trypanosomes are thus dealt with separately below.

(A) BOVINE TRYPANOSOMIASIS DUE TO *T. BRUCEI*.

An opportunity to test out the efficacy of Antimosan against *T. brucei* infection of bovines has not yet presented itself. It is, however, not anticipated that Antimosan treatment would prove to be a failure in the treatment of this disease in bovines for the reason that *T. brucei* infection of horses, which is much more severe in its effects in horses than in bovines, has shown good response to treatment with this drug.

(B) BOVINE TRYPANOSOMIASIS DUE TO *T. VIVAX*.

For this experiment five bovines were utilized. These were infected by sub-inoculation from an infected bovine on the 10th January, 1929, by the injection of 10 c.c. blood subcutaneously. In no case was there any failure in the transmission. Diagnosis was chiefly centred on the examination of lymphatic gland smears. Unfortunately there was transmitted over at the same time Piroplasmosis and Anaplasmosis with the result that the animals lost condition rapidly and had to receive treatment for these diseases. One of the experimental animals died from the Anaplasmosis.

Table I gives the details in connection with this experiment. The Antimosan in every case was given subcutaneously in a 12 per cent. solution.

TABLE I.

T. VIVAX *Antimosan Therapy.*

Bovine.	Date of Infection.	Complications.	Treatment Commenced.	Weight of each Dose.	Number of Doses.	Intervals.	Remarks.
2715	10/1/29	Piroplasmosis Anaplasmosis	31/1/29	3 gm.	1	—	5/2/29 died Anaplasmosis.
2765	,,	,,	12/3/29	1·8 ,,	3	8 days	Sterilization. No tryps. found to 27/5/29.
2766	,,	,,	4/3/29	1·8 ,, 1·2 ,, 1·8 ,,	2 2 1	8 days	Sterilization. No tryps. found up to 27/5/29.
B 2743*	,,	,,	—	1·2 ,, 1·8 ,, 3·0 ,,	1 1 1	8 days	Sterilization. No *T. vivax* found up to 27/5/29.
B 2727	,,	,,	Control	—	—	—	Tryps. were found up to 28/8/29.

* This bovine was found to have become infected with *T. congolense* just before treatment.

DISCUSSION.

Notwithstanding that the bovines in this experiment lost condition rapidly on account of the intercurrent affections, yet they showed no constitutional disturbance as a result of the injection of the Antimosan. It was not expected that the mortality among these animals would be high for our experience has been that, if bovines suffering from *T. vivax* disease are kept under good conditions, the disease rarely proves fatal. The one animal that died in this experiment had an extremely severe attack of Anaplasmosis.

On account of the comparatively minor importance of *T. vivax* infection and the success which was obtained in the above preliminary trial of Antimosan in the treatment of *T. vivax* disease, it was decided not to institute further trials but rather to concentrate on the more important and urgent problem of *T. congolense* infection.

CONCLUSION.

(1) Of the four bovines treated with Antimosan, three were apparently sterilized and one died of Anaplasmosis.

(2) The control showed *T. vivax* for a period of 230 days.

(C) BOVINE TRYPANOSOMIASIS DUE TO *T. CONGOLENSE*.

The Antimosan therapy of bovine trypanosomiasis caused by *T. congolense* is dealt with below under two headings namely (1) Short Interval Treatment, and (2) Long Interval Treatment. By the former is understood treatment by the administration of Antimosan at intervals of 8 days or less and by the latter at intervals of 28 days.

(1) Short Interval Antimosan Theraphy.

The treatment of trypanosomiasis in general has been almost entirely on the lines of the administration of the selected drug on consecutive days, or at intervals of a few days at the most. Consequently such short interval methods of administration were followed in the first trials of the efficacy of Antimosan in *T. congolense* infection of bovines.

(*a*) *Preliminary Experiment.*

For the first experiment five bovines were utilized. These bovines were infected by the subcutaneous injection of 5 c.c. citrated guinea-pig blood rich in *T. congolense*. In no case was there a failure in the transmission. Diagnosis was chiefly centred on the examination of blood smears. Table II gives the details in connection with this preliminary trial. The Antimosan in every case was given subcutaneously as a 12 per cent. solution.

Table II.

T. congolense. *Short Interval Treatment.*

Bovine.	Date of Infection.	Complications.	Treatment Commenced.	Weight of each Dose.	Number of Doses.	Intervals.	Remarks.
2634	10/1/29	—	3/2/29	1·2 gm.	5	8 days	Unsuccessful.
2639	,,	Anaplasmosis	23/1/29	3 ,,	5	8 days	Sterilization.
2702	,,	—	8/2/29	3 ,,	2	8 days	,,
2709	,,	Anaplasmosis	16/2/29	1·2 ,,	5	4 days	Unsuccessful.
2714	,,	—	Control	—	—	—	Smears + after 5 months.

Discussion.—As a result of the treatment bovine 2634 showed a marked decrease of the severity of the disease for a period of 10 weeks when the trypanosomiasis again commenced to show acute exacerbations. During treatment the trypanosomes were found during all the intervals between injections. Bovine 2709 showed results very similar to those of 2634 but acute exacerbations did not become evident. The ultimate result of treatment in this animal even though it was a failure as far as sterilization was concerned was excellent. Its anaemia disappeared entirely, the red cell count returning to normal, its condition became good and trypanosomes became difficult to find. Bovines 2639 and 2702 were both sterilized. This was proved by negative blood smear examinations over a period of 9 and 10 weeks respectively, by sub-inoculation each into two susceptible bovines and by the test for determination of sterility to be described later.

Conclusions.—(1) Subcutaneous injections of 3 gm. Antimosan repeated twice and five times at intervals of 8 days resulted in sterilization.

(2) Subcutaneous injections of 1.2 gm. Antimosan repeated five times at intervals of 8 and 4 days gave negative results.

(*b*) *Antimosan at intervals of 7 days.*

As a result of the above experience, further bovines were submitted to treatment. Details are tabulated on Table III. Of these five bovines two, namely 2634 and 2714, were one failure and the control respectively of the previous experiment and one, 2743, was brought forward from the *T. vivax* experiment. This latter animal had become stable infected with *T. congolense* just prior to the treatment which was successful against the *T. vivax* infection, but unsuccessful against the *T. congolense* infection. The time of infection in each case is the date of the original infection with the exception of 2743, where the date indicates the first detection of *T. congolense* in the blood-smears.

TABLE III.

T. CONGOLENSE. *Short Interval Treatment (7 days).*

Bovines.	Weight in Kg.	Date of Infection.	Treatment Commenced.	Each Dose in Gm.	Number of Doses.	Dose per Kg.	Remarks.
						Gm.	
2634	238	10/1/29	26/6/29	3	2	0·013	Sterilization. Negative smears for 11 weeks.
2714	231	10/1/29	26/6/29	3	2	0·013	Do. do.
2464	386	20/4/29	20/4/29	3	2	0·008	Unsuccessful. Tryps. found 7th week.
2468	375	20/4/29	20/4/29	3	2	0·008	Unsuccessful. Tryps. found 8th week.
2743	181	5/4/29	20/4/29	3	2	0·017	Unsuccessful. Tryps. found 3rd week.

Chart I illustrates the sterilization of bovine 2634 by two doses of Antimosan at a seven-day interval.

Discussion.—It is of interest to note that bovine 2634, which had not been sterilized by the previous treatment of 1.2 gm. repeated 5 times yet became sterilized by the administration of the same total quantity divided into two doses, whereas bovine 2743 which was of less weight and which failed (see Table I) to an administration of 6 gm. given as 1.2 gm., 1.8 gm., and 3 gm. again failed to a total of 6 gm. divided into two doses. There would appear to be a possibility of the production of antimony-fast trypanosomes in this animal. Yet it is remarkable that a single dose of 6 gm. resulted in sterilization of this animal (see Table IV). The two bovines 2464 and 2468 considerably heavier than the others did not become sterilized. However, there was a marked depression of the virulency of the trypanosomes, the anaemia practically disappeared and the condition of the animals improved. The greater weight of these two probably was a factor in the failure of the treatment.

Conclusions.—Antimosan in a 3 gm. dose repeated at an interval of one week produced sterilization in two out of five bovines. Of the failures two were decidedly heavier than the successful cases and one had been submitted to increasing doses previously.

(c) Antimosan at intervals of less than seven days.

To enable a saving of time to be brought about in the treatment when the object is to obtain rapid sterilization of bovines, for example, when it is desired to remove bovines from an infected to a non-infected area, it was decided to test out somewhat larger doses than had previously been employed, or moderate doses repeated on consecutive days. The results obtained are tabulated in Table IV. As previously, the date of infection of bovine 2743 indicates the date on which *T. congolense* was first found in blood smears, whereas in the other three bovines it indicates the date of inoculation.

TABLE IV.

T. CONGOLENSE. *Short Interval Treatment.*

Bovine.	Weight in Kg.	Date of Infection.	Treatment Commenced.	Each Dose in Gm.	Number of Doses.	Dose per Kg.	Remarks.
2743	236	5/4/29	16/10/29	6	1	0·025	Sterlization. Negative smears to 51st day.
3627	318	13/9/29	16/10/29	6	1	0·019	Unsuccessful.
3542	204	13/9/29	24/10/29	6	1	0·029	Unsuccessful.
3527	218	13/9/29	24/10/29	3	2	0·014	Sterilization. Negative smears to 71st day.

Discussion.—The results were not sufficiently promising to justify the continuation of experimentation along these lines especially as constitutional disturbances were noted in bovine 3542 and 3527. Alternative methods of treatment with a greater expenditure of time have proved to be so successful that it is not considered that the above procedure would develop into a method of treatment which could be recommended for practical application. It is worth while again to note that sterilization of bovine 2743 resulted with a dose of 6 gm. whereas two previous failures had occurred with a similar dosage administered as three ascending and two equal doses. This animal was intermediate in weight to 3627 and 3542, the two failures. It, however, had been infected for approximately 150 days longer. The variation in response to treatment of bovines infected for varying periods will be referred to again in this article.

Conclusions.—(1) The administration of more massive single doses of Antimosan to obtain immediate sterilization was not of sufficient promise to justify, at this stage, the continuation of the experimentation.

(2) Similar conclusions were arrived at in connection with the administration of Antimosan on consecutive days.

Summary and Conclusions of Short Interval Treatment.

The lines of experimentation in the foregoing treatments of bovine trypanosomiasis caused by *T. congolense* have followed more

or less the lines generally advocated in the treatment of trypanosomiases in general. By the time these experiments on Short Interval Treatment had reached this stage, considerable advance had been made in the experiments in connection with the Long Interval Treatment. As this latter held out such great promise in the surmounting of the problem of bovine trypanosomiasis all facilities available were devoted to its study. The experimentation on the Short Interval Treatment consequently was discontinued.

As far as the experiments went, however, it would appear that a dose of 3 gm. of Antimosan given twice at weekly intervals would be insufficient to bring about sterilization in bovines above 250 Kg. in weight. The indication is that the dose should be increased or perhaps preferably repeated more often. Even for bovines lighter than 250 Kg. this treatment might prove unsuccessful.

(2) Long Interval Antimosan Therapy.

Until such time that methods are developed and instituted to destroy the tsetse fly, the sterilization of bovines of trypanosomes which have to continue to live near or in tsetse areas is of problematic advantage. Such sterilized bovines become susceptible again and would consequently have to resist further attacks of the disease. The sterilization of bovines has not proved a difficult undertaking but no lasting benefit can be looked for unless such bovines are removed entirely from further contact with infection. If a true immunity were to result after recovery, such sterilization would be of considerable benefit, but no evidence of such immunity has been obtained throughout this work.

Consequently, when bovines have to live in or near tsetse fly belts, some substitutional method of control must be devised. The treatment already described under Short Interval Treatment results in sterilization, but it is manifestly impossible on account of expense of drugs and of handling to be continually treating bovines by that method. It was conceived that this difficulty might be overcome by utilizing a long interval routine method, a method which would entail a minimal amount of handling and a small annual expenditure on drugs.

It is a well known fact that the treatment of trypanosomiasis with arsenic compounds has always been on the principle of the administration of large doses with the object of destroying the trypanosomes as quickly as possible. The danger otherwise is that drug-fast trypanosomes may be developed. Somewhat similar conditions are held to govern the treatment of trypanosomiasis with antimony compounds.

Bearing in mind these points, it can be seen that the institution of experiments for the object of observing the effect of doses of an antimony compound given at long intervals appeared to be scarcely justified, especially after the failures recorded in the treatment of Bovine 2743 already referred to.

Notwithstanding this, such experiments were decided on, not with the idea that sterilization would be obtained, but for the purpose of determining whether by such means bovines could be enabled to survive in tsetse fly areas. It was fully expected that such a

procedure would result in the production of drug-fast trypanosomes, but it was hoped that a method might be obtained whereby the trypanosomes are caused to disappear for a period in between the administration of the drug. Such disappearance or partial control of the disease would then free the animal for part of the time at any rate from the detrimental effects of the parasite and enable it thus to survive in infected areas. Furthermore, it was argued that even though non-sterilizing doses at short intervals might result in the production of drug-fast parasites, yet it did not necessarily follow that the administration of a similar dose at longer intervals would so result.

Very great practical importance would be attached to the discovery of a successful routine treatment which would necessitate the handling of bovines, constantly exposed to infection, only at long intervals and which would involve a comparatively small outlay on drugs during a year.

It is of interest to note here that Professor Browning, of the University of Glasgow, forwarded to the Director of this Institution, under cover of a letter dated 2nd September, 1929, a reprint of an article by himself and co-workers (1929) in which the following passage occurred:—" While resistance can be acquired by trypanosomes subjected to increasing doses of the drug (a styryl compound), it is of interest that when in a particular animal each of successive relapses is treated by the same moderate dose, drug resistance does not tend to develop. In fact it has been shown that cure may ultimately be effected by this procedure."

(*a*) *Preliminary Experiment.*

The details of the experiment are tabulated in Table V. The arrangement of this experiment was to give 3 gm. doses of Antimosan at intervals of four weeks to bovines infected with *T. congolense*. To control the results, temperatures were taken twice daily and blood-smear examinations were carried out three times weekly. By the period of relapse is understood the number of days that elapsed after the last injection of Antimosan before trypanosomes reappeared in the blood.

TABLE V.

T. CONGOLENSE. *Long Interval Treatment* (28 *days*).

Bovine.	Weight in Kg.	Date of Infection.	Treatment Commenced.	Each Dose in Gm.	Dose per Kg.	Periods of Relapse.					Remarks.
					Gm.						
2471	354	20/4/29	26/6/29	3	0·008	16	26	0	0	0	Sterilization.
2473	486	20/4/29	26/6/29	3	0·006	16	0	0	0	0	,,
3502	146	26/6/29	10/7/29	3	0·021	8	0	0	0	0	,,
3520	191	26/6/29	10/7/29	3	0·016	12	14	26	0	0	,,

Chart II illustrates the sterilization of bovine 3502 by two doses of Antimosan at a four-week interval.

Discussion.—The excellent results obtained in the above experiment were not anticipated. The ultimate results were, however, indicated by the remarkable fact of the lengthened period of relapse in those cases in which the trypanosomes did not disappear after the second injection. The uniformly successful outcome was obtained in spite of the fact that the bovines used varied considerably in weight. Bovine 2473 was two and a half times the weight of Bovine 3520, yet a similar dose of Antimosan brought about a trypanosome-free interval sooner in the former than in the latter. It would appear that there is some factor present which results in quicker response to treatment by certain animals. It is the writer's opinion that the period of time that elapses after infection before treatment is instituted has a bearing on this matter. This aspect will be dealt with during the course of this chapter.

(*b*) *Continuation and Modification of Preliminary Experiment.*

To support the results obtained in the previous experiment, further bovines were submitted to the four weekly administration of Antimosan. In addition, a further modification of the treatment was carried out by reducing the amount of Antimosan injected from 3 gm. to 1.5 gm. By this means it was hoped to obtain results which, although not comparable to those obtained by the use of the 3 gm. dose, yet would bring about an appreciable reduction in the expenditure on the drug, perhaps with not too great a decrease of efficacy. The results are tabulated in Table VI.

TABLE VI.

T. CONGOLENSE. *Long Interval Treatment* (28 *days*).

Bovine.	Weight in Kg.	Date of Infection.	Treatment Commenced.	Each Dose in Gm.	Dose per Kg.	Periods of Relapse.			
					Gm.				
416	413	20/4/29	16/10/29	3	0·007	0	0	0	—
3524	166	26/6/29	16/10/29	3	0·018	0	0	0	0
2639	257	26/6/29	16/10/29	3	0·012	0	0	0	—
2464*	425	17/10/29	20/11/29	3	0·007	12	12	0	0
2634*	300	17/10/29	20/11/29	3	0·01	7	0	0	0
2468*	381	17/10/29	20/11/29	1·5	0·004	7	9	0	19
2994	218	17/10/29	20/11/29	1·5	0·007	9	9	0	0
3527	227	13/9/29	20/11/29	1·5	0·007	12	21	0	0
3627	318	13/9/29	20/11/29	1·5	0·005	12	9	9	0

* These three bovines were premune to one strain when re-infected with another strain on 17/10/29.

Discussion.—The 3 gm. dose of Antimosan given at four-week intervals showed great efficacy in the three first tabulated bovines, the first administration in each case producing a trypanosome-free interval. In the remaining two such a period was not obtained until the third and second administration.

The results obtained by the administration of 1.5 gm. Antimosan at four-week intervals would appear to be quite promising. If in conjunction with the table, the temperature charts, of which Charts

III and IV are included below as typical examples, are considered, it would appear that a comparatively small dose has a quite decided effect in controlling the disease. In another as yet uncompleted experiment, it has been shown that a bovine submitted to the Long Interval Treatment, using a dose of 3 gm. Antimosan, can be re-infected during the interval by the injection of the homologous strain of *T. congolense*. Consequently, a bovine constantly exposed to infection would most likely, as far as the homologous strain is concerned, alternate between a state of sterility and a state of premunition. The use of a dose of 1.5 gm. Antimosan in the circumstances would probably be indicated, as by its means a somewhat similar alternation of sterility and premunition would be arrived at with a lessened expenditure on drugs. The practical value of the decrease in expenditure so brought about does not need emphasis.

Conclusions.—(1) The results with the 3 gm. dose of Antimosan support those obtained in previous experiments.

(2) A dose of 1.5 gm. Antimosan at similar intervals appears to be decidedly promising for practical application under field conditions.

Summary and Conclusions of Long Interval Treatment.

Sterilization of bovines infected with *T. congolense* is obtained by the subcutaneous injection of 3 gm. Antimosan when given at four-weekly intervals. In some cases this object is attained after the first injection. If not there is a gradual lengthening of the time before reappearance of the trypanosomes is noted until ultimately a trypanosome-free period of four weeks is obtained. This sterile condition is lost on re-infection of the bovine with a homologous strain. Consequently, under field conditions, one would expect bovines at times to alternate between sterilization and premunition. The latter state results when a bovine shortly after the Long Interval Treatment is infected with a homologous strain. Cessation of a treatment which has produced either premunition or sterilization results consequently in an area where the bovine is constantly exposed to re-infection in premunition against the strains of *T. congolense* which have previously infected it. The writer is not at present in a position to say that such a premunition would be, under unfavourable conditions, sufficiently effective to enable the animal to survive. The indications are that it would not be.

The use of a smaller dose of Antimosan may possibly be as efficacious in its ultimate results as far as the control of bovine trypanosomiasis is concerned as a larger dose. There was noticed, in connection with the Long Interval Treatment and to a less extent, with the Short Interval Treatment, a certain amount of variation in the response to the injection of Antimosan. This variation could not be entirely ascribed to the weight factor. Tabulation as in Table VII seems to indicate that the variation is correlated to a certain extent with the varying length of time which was allowed to elapse after infection before treatment was instituted. The date of infection of bovines infected with one strain and then infected with a different strain is entered in the table as under the date of infection with the latter.

Table VII.

T. congolense. *Variation in Response to Treatment.*

Bovine.	Infection to Treatment in Days.	Weight in Kg.	Treatment.	Periods of Relapse.			
3502	14	146	*L.I.T. 3 gm. doses.....	8	0	0	0
3520	14	191	,, ,, ...	12	14	26	0
2464	34	425	,, ,, ...	12	12	0	0
2634	34	300	,, ,, ...	7	0	0	0
2471	67	354	,, ,, ...	16	26	0	0
2473	67	486	,, ,, ...	16	0	0	0
3524	112	166	,, ,, ...	0	0	0	
2639	112	257	,, ,, ...	0	0	0	
416	179	413	,, ,, ...	0	0	0	
2468	34	381	,, 1·5 gm. doses.....	7	9	0	19
2994	34	218	,, ,, ...	9	9	0	0
3527	68	227	,, ,, ...	12	21	0	0
3627	68	318	,, ,, ...	12	9	9	0

* L.I.T. = Long Interval Treatment.

The response to treatment is judged by the number of injections of Antimosan necessary to bring about a trypanosome-free interval. Those animals which were infected for a long time before treatment was commenced gave such an interval early, notwithstanding that the weights of these were well up to the average. Where the smaller dose of 1.5 gm. was used, this variation in response was not as well brought out on account of the variation in the time interval not being so great as in the case of the treatment with the 3 gm. dose.

Somewhat similar conclusions were arrived at in connection with the application of the Short Interval Treatment. Tables VIII and IX illustrate the influence of late treatment when 3 gm. and 6 gm. doses were used. These two groups represent animals which have received identical treatment.

Table VIII.

Bovine.	Infection to Treatment in Days.	Weight in Kg.	Treatment.	Result.
2702	29	220	*S.I.T. 3 gm. 2 doses.....	Sterilization.
2464	67	220	,, ,, ...	Unsuccessful.
2468	67	220	,, ,, ...	Unsuccessful.
2743	82	181	,, ,, ...	Unsuccessful.
2634	168	238	,, ,, ...	Sterilization.
2714	168	231	,, ,, ...	Sterilization.

* S.I.T. = Short Interval Treatment.

TABLE IX.

Bovine.	Infection to Treatment in Days.	Weight in Kg.	Treatment.	Result.
3627	33	318	*S.I.T. 6 gm. 1 dose.....	Unsuccessful.
3642	41	204	„ „	Unsuccessful.
2743	194	236	„ „	Sterilization.

* S.I.T. = Short Interval Treatment.

In Tables VIII and IX there appears to be an influence other than that of weight of the bovine. Greater success was obtained when the period before treatment was long.

There is scarcely sufficient evidence, of the bovines grouped in Tables VII, VIII and IX, to permit one to dogmatise but it undoubtedly appears that the response to treatment is influenced considerably by the time that elapses between infection and treatment. This point should prove to be of great value and importance in the treatment of bovine trypanosomiasis in the field. The success obtained in these experiments with late treatment will enable an easier sterilization to be attained in bovines which have been suffering from the disease for a long time, a state very likely to be encountered in undeveloped areas. A possible disadvantage of the Long Interval Treatment is that an infected animal might be exposed to adverse conditions of such a severity that the first dose given might be insufficient, under such conditions, to carry it over to the end of the four-week interval when its second dose becomes due. This factor is not likely to be of influence if the bovine has received a number of doses as it would have, by then, undoubtedly developed a certain degree of resistance. However, if the conditions were such that there was danger that the first dose would not carry the bovine over to the second, a shorter interval between first and second dose could be employed. A few short interval doses before the institution of the Long Interval Treatment is not likely to interfere with the results obtained. Such a procedure is at present under trial in the field. Reports so far indicate that this modification is proving satisfactory.

Cheapening of the treatment of bovine trypanosomiasis will, as noted above, probably be brought about by decreasing the dose. It may even be possible to further decrease expenditure by increasing after the first few doses, the length of the intervals. If one were dealing with only one strain of *T. congolense* no danger in introducing such an increase would be anticipated. However, one should be careful of increasing the length of the interval to too great an extent, especially if conditions are unfavourable, on account of the danger of the bovines concerned becoming infected with a heterologous strain of *T. congolense* and succumbing to this strain before the time for injection of the next dose comes around, because

resistance to re-infection is only developed in a bovine under treatment to the homologous strain. This danger, however, should not be of great moment if the animals be kept under close observation by an intelligent person. Any clinical manifestations of bovine trypanosomiasis would call for the administration of a dose before its pre-arranged time is due.

As far as our present knowledge of the strains of *T. congolense* goes, it is indicated that the administration of Antimosan should be carried out in doses of 1.5 gm. to 3 gm. at regular not too long intervals to protect the bovines against the heterologous strains. It should also be remembered that, if a bovine is sterilized of a particular strain of *T. congolense*, it again, in the temporary absence of this strain, becomes liable to an acute attack of bovine trypanosomiasis on the return of this strain.

No indication of the production of antimony-fast trypanosomes by the use of a 3 gm. of Antimosan have been met with. The experiments in connection with the use of the 1.5 gm. dose of Antimosan although just completed, also indicate that no such antimony-fast trypanosomes are produced.

IV. DETERMINATION OF STERILITY.

Throughout this work a difficulty which hampered considerably its advancement was that of arriving at a decision as to whether any particular animal was sterilized of the trypanosomes or still retained them. In some cases negative smear examinations were obtained over a long period, the animal itself was in good health and blood examinations showed no anaemia, yet one had no reason, on account of experience obtained during the experiments, to believe that they did not harbour parasites. In other words they were in such a high state of premunition that the only abnormality apparently was the occasional detection of a trypanosome. As an example of the above type may be mentioned bovine 2709. During a period of eleven weeks of blood smear examination carried out three times weekly, trypanosomes were only found on four occasions, on three of these occasions only one being found after prolonged search.

As sub-inoculation into susceptible bovines to prove sterilization would prove an expensive procedure, a method, cumbersome, no doubt, but more economical, was devised. This consisted in the re-inoculation of the bovines, which it was desired to test, with guinea-pig blood rich in trypanosomes of the same strain of *T. congolense*. Naturally of course, this method is only of assistance under conditions of experimentation where it was desired to determine the effectiveness of treatment without the laborious process of blood smear examinations over a long period.

A disadvantage of this method is that it is necessary to permit a certain period of time to elapse after the completion of the treatment to enable the bovines to get rid of the resistance to re-infection which is always present after recovery from the disease. A bovine which has been sterilized by treatment reacts in one of two ways. Either it reacts as if it were a bovine which had not had an attack of the disease previously, i.e. after a short incubation period

trypanosomes become numerous, there is a thermal reaction and anaemia develops, or it drops directly into a state of premunition. Whether it reacts as in the first or second case depends entirely on the period of time that is permitted to elapse after sterilization. The writer is not at present in a position to state definitely the length of the period of time necessary to bring the reaction under the first mentioned heading, but it is certainly less than 152 days and more than 52 days.

TABLE X.

Test for Determination of Sterility.

Bovine.		Days after Inoculation.																
		4	5	6	7	8	10	11	12	13	14	15	17	18	19	20	21	21
2702	Sterile.....	—	—	—	—	1+	1+	4+	5+	3+	3+	3+	3+	3+	2+	2+	1+	2+
2639	,, ...	—	—	—	—	1+					3+	1+	2+	1+				1+
2634	Presumed Sterile....	—	—	—	—	—	—	—	2+	2+	1+	—	—	—	—	—	—	—
2714	,, ..	—	—	—	—	—	—	—	—	—	—	—	—	1+	—	—	—	—
2464	Premune ...	—	—	—	—	—	2+	1+	3+	2+	—	—	1+	2+	2+	—	1+	1+
2468	,, ...	—	—	—	—	—	—	—	—	—	—	—	1+	—	—	—	—	—
2743	,, ...	3+	—	—	—	1+	1+	2+	3+	2+	2+	—	—	—	—	3+	2+	—
2709	,, ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3506	Presumed non-infected..	—	—	—	1+	2+	4+	2+	2+	4+	2+	2+	3+	1+	2+	3+	3+	1+
3525	,,	—	—	2+	2+	3+	1+	2+	4+	2+	1+	2+	3+	1+	3+	3+	3+	1+
3524	Newly infected..	4+	3+	—	1+	3+	2+	2+	3+	3+	2+	2+	1+	2+	4+	4+	3+	4+
416	Chronic.....	—	2+	3+	—	—	—	—	—	—	1+	2+	—	—	—	—	—	—
3627	Control.....	1+	2+	2+	4+	4+	3+	4+	4+	2+	3+	2+	2+	3+	3+	3+	2+	3+

Trypanosomes absent (—), very raro (1+), rare (2+), frequent (3+), very frequent (4+).

Explanation of Table.

The two bovines 2702 and 2639 had been proved to be sterile by negative blood smear examinations over periods of 18 and 17 weeks respectively, and by sub-inoculating 100 c.c. blood from each into two bovines. These four bovines then in turn were submitted to blood smear examinations and clinical observations for 13 weeks, in addition to the test now under discussion, with negative results.

The two bovines 2634 and 2714 classified as " presumed sterile " are animals which were treated and which, for a period of 11 weeks, gave negative results to blood smear examinations.

The four " presumed non-infected " bovines 3506, 3525, 3527 and 3542 are bovines which had been used to test by sub-inoculation the sterility of bovines 2639 and 2702 referred to above.

For purposes of comparison there are also introduced four " premune " bovines 2464, 2468, 2743 and 2709, one bovine 3524, recently infected, one chronic case, 416, and one control 3627.

CHART I.

D. 2634 T CONGOLENSE.

ANTIMOSAN IN DOSES OF 3 gm AT A SEVEN-DAY INTERVAL

CHART II.

B. 3502. T. CONGOLENSE.

ANTIMOSAN IN DOSES OF 3 gm. AT A FOUR-WEEK INTERVAL

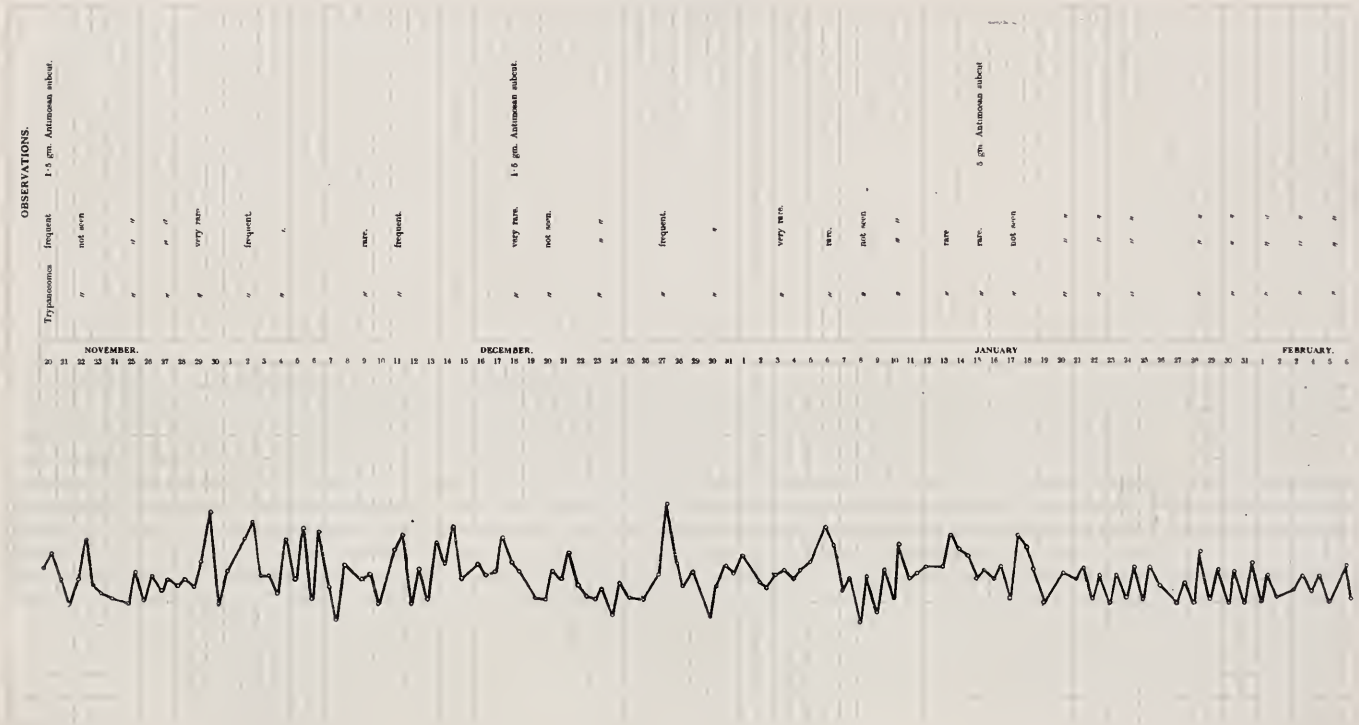
CHART III.

B. 2468. T. CONGOLENSE.

CONTROL WITHOUT STERILIZATION BY THE USE OF 1·5 gm. DOSES OF ANTIMOSAN AT FOUR-WEEK INTERVALS.

CHART IV.

B. 2994. T. CONGOLENSE.



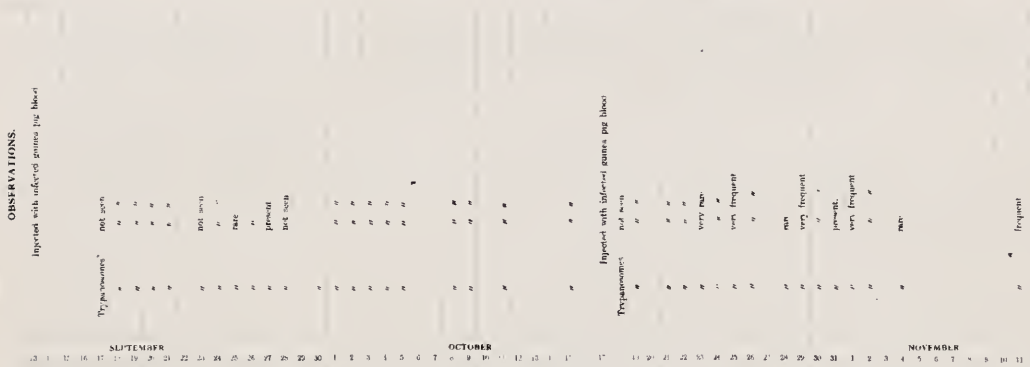
CONTROL WITH STERILIZATION (?) BY THE USE OF 1·5 gm. DOSES OF ANTIMOSAN AT FOUR-WEEK INTERVALS.

CHART V.

BOVINE 2702. T. CONGOLENSE.

CHART VI.

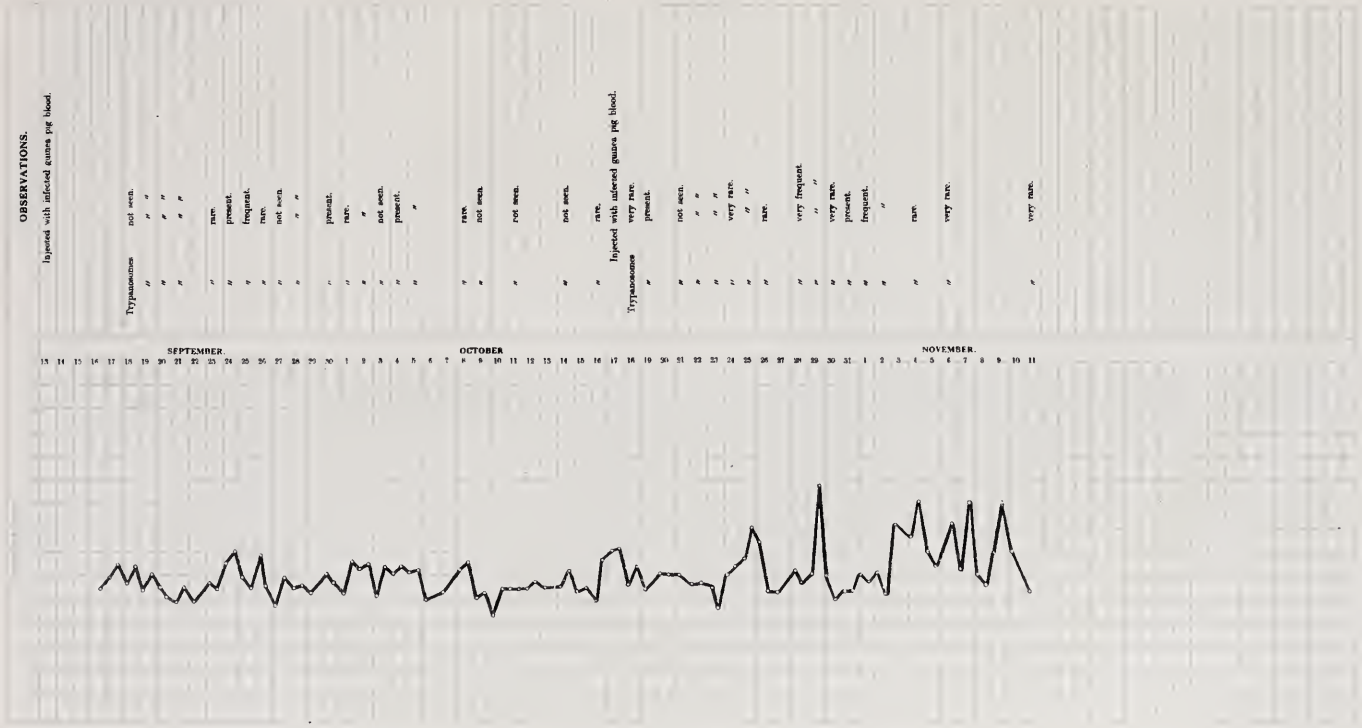
BOVINE 2634. T. CONGOLENSE



THE EFFECT ON A 52-DAY STERILISED BOVINE OF THE INJECTION OF THE HOMOLOGOUS STRAIN AND SUBSEQUENTLY OF A HETEROLOGOUS STRAIN.

CHART VII.

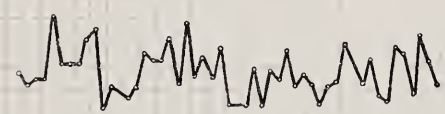
B. 2464. T. CONGOLENSE.



THE EFFECT ON A PREMUNISED BOVINE OF THE INJECTION OF THE HOMOLOGOUS STRAIN AND SUBSEQUENTLY OF A HETEROLOGOUS STRAIN.

CHART VIII.

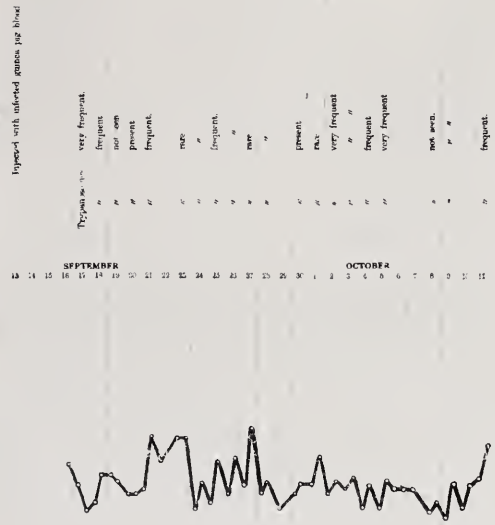
BOVINE 3525. T. CONGOLENSE.



THE EFFECT OF THE INJECTION ON A PRESUMED NON-INFECTED BOVINE.

CHART IX.

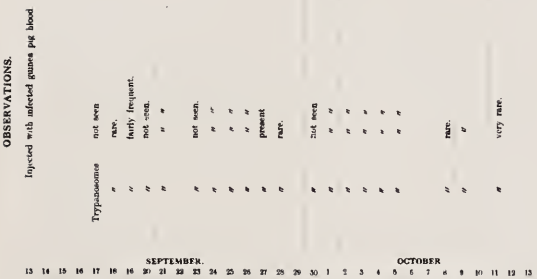
BOVINE 3524. T CONGOLENSE.



THE EFFECT ON A RECENTLY INFECTED BOVINE OF THE INJECTION OF THE HOMOLOGOUS STRAIN.

CHART X.

BOVINE 416. T. CONGOLENSE.



THE EFFECT ON A CHRONIC CASE OF THE INJECTION OF THE HOMOLOGOUS STRAIN.

CHART. XI.

BOVINE 3627. T. CONGOLENSE.

CONTROL FOR STRAIN USED AS HOMOLOGOUS.

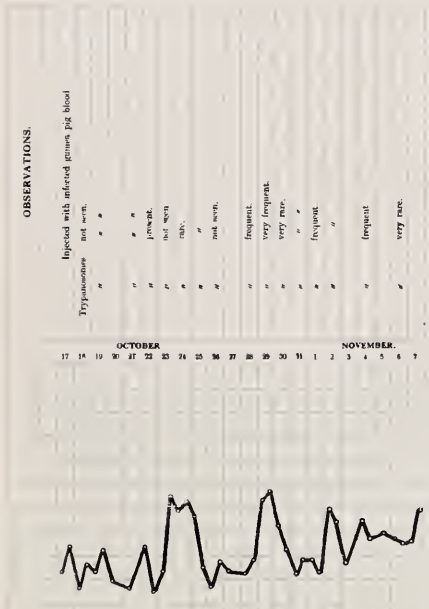
OBSERVATIONS.

Injected with infected guinea pig blood

Trypanosomes	present.
"	rare.
"	"
"	very frequent.
"	" "
"	frequent.
"	fairly frequent.
"	" "
"	rare.
"	frequent
"	rare.
"	rare.
"	frequent.
"	"
"	"
"	rare.
"	frequent
"	very rare
"	frequent
"	frequent.

CHART XII

BOVINE 2994. T. CONGOLENSE.



CONTROL FOR STRAIN USED AS HETEROLOGOUS.

Charts.

The charts V to XI are representative examples of the temperature reactions obtained by the injection of the test inoculum of the guinea-pig blood containing the particular strain of *T. congolense*.

Discussion.

By examination of the table and the charts it will be seen that the bovines can be classified according to the occurrence of the trypanosomes and the thermal reaction into three groups.

Group I.—This one includes the two "sterile," all of the "presumed non-infected" and the "control." They all behaved as if they were receiving infection for the first time.

Group II.—In this group are the two "presumed sterile" and the "premune." A variation among these is the very early appearance in one premune bovine of trypanosomes. The irregularity in connection with the re-infection of the two "presumed steriles" as compared with the two "steriles" of Group I is due to their greater resistance on account of this test having been carried out at a comparatively short time after their sterilization. By this test alone, therefore, it is not possible to determine sterility unless the bovines to be tested be retained for a period longer than these were namely 52 days, before submitting them to the test.

Group III.—Includes the "newly infected" and the "chronic" types. In neither of these could any change be detected. In addition, bovines of this group showed more frequent trypanosomes and greater irregularity of temperature than those of Group II.

Conclusions.

(1) A test for determination of sterility is described which has proved to be of some assistance when it is necessary to determine whether a bovine has been sterilized or not as the result of treatment.

(2) A necessary essential to the proper working of the test is that sufficient time be allowed to elapse before carrying out the test to permit of the disappearance of the resistance to re-infection which is always present for some time after sterilization.

(3) If such a period is not allowed, there arises the difficulty of differentiating between such a resistant sterile bovine which, after test, may only show occasional trypanosomes and a strongly premune one which, whether tested or not, will show trypanosomes often only at long intervals.

V. IDENTIFICATION OF AND PREMUNITION AGAINST VARIOUS STRAINS OF T. CONGOLENSE.

In the article by Parkin and Hornby (1930), it was shown that the premunition of bovines against a particular strain did not protect them from reacting to a heterologous strain of *T. congolense*. The object thus to strive for in any method of practical worth for premunising of bovines, to enable them to survive in tsetse fly areas, should be to obtain premunition against all the strains of this trypanosome occurring in the particular area. But we are at

present not in a position to say how many strains of *T. congolense* there are in the various areas. Consequently, before any method of premunition can be attempted by laboratory means, it is necessary that a method of identification of the various strains be devised. Once such knowledge is available it should not be an insurmountable obstacle to preserve these strains at a laboratory and to bring about premunition of bovines by means of inoculation and subsequent treatment in a manner somewhat similar to that described elsewhere in this article. A complicating factor undoubtedly would be the discovery that the number of strains of *T. congolense* was very numerous.

It was found during the course of this work, that a bovine premunised against a particular strain reacted to a different strain, as if it were being infected for the first time. This reaction is judged by the temperature curve, the occurrence and multiplication of the trypanosomes and the onset of anaemia and is well illustrated on Charts IV and V with the Control Chart XII.

Although only two strains were available for this work, it is considered permissible to draw the conclusion that further heterologous strains would behave in a similar manner.

To enable, therefore, a fairly rapid identification of the various strains of *T. congolense* to be carried out, all that would be necessary would be the retention of bovines which were in a state of premunition against the strains of *T. congolense* already identified, and the inoculation of these bovines wth the strain which it was desired to identify. A reaction would indicate the presence of a new strain whereas no reaction would indicate that the strain was one which had already been identified. The particular bovine used for the inoculation, if it reacted, would then have to be brought to a state of premunition against the newly identified strain. Once this is attained the bovine would be ready for use for the identification of further strains. If the bovine did not react it would be available immediately for this purpose. The same bovine could be used time after time serving the double purpose of a reservoir of all the strains identified and a test animal for inoculation of newly collected trypanosomes.

At times the reaction obtained in the test bovines by the inoculation of new strains would be severe. It becomes necessary, therefore, to consider the effect of treatment, which it might be necessary to institute, in some of these cases, for the purpose of controlling the reaction and, incidentally, of bringing the bovines into a state of premunition against the newly introduced strain. In another part of this article it is pointed out that bovines, which have been infected with *T. congolense* for a considerable time, respond to treatment very much better than those infected for only a short time. Consequently the test premunised bovines, if treatment be instituted to control the reaction of the newly introduced strain, would be in danger of losing the infection by the strains which were first introduced. If this were to happen they would become of course after a time fully susceptible to these strains again and then would react as if the strains had not previously been used. This would lead to a certain amount of re-identification. If, however, a bovine

recently sterilized be inoculated with the same strain it passes, without showing any reaction, directly into the state of premunition. It becomes necessary, therefore, to re-inoculate occasionally the bovines, used for test purposes, with the strains already identified. This re-inoculation is specially necessary after treatment has been carried out.

One must ultimately get to a stage in the identification of the various strains which may be few or many, when all the strains of *T. congolense* are collected in one or more bovines. From these reservoirs can then be obtained blood infected with the various strains which could be used for setting up the disease in bovines which were to be premunised. The reaction in these would be controlled by treatment. To make certain that all the strains have survived in the inoculated bovine, a second injection from the reservoir would have to be given. The inoculated bovines would then be premune against all the strains of *T. congolense* identified.

However, there possibly will not arise any necessity for the introduction of this system of premunition, except under exceptional circumstances, if the method described elsewhere in this article stands the test of field conditions.

Conclusions.

(1) A method of identification of the various strains of *T. congolense* is described.

(2) A method of artificial premunition of bovines against various strains of *T. congolense* is described.

I should like to express my appreciation to the Director, Dr. P. J. du Toit for the advice and encouragement freely given at all times, and also to acknowledge the assistance I have received from Dr. E. M. Robinson.

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Antimony Therapy in Equine Trypanosomiasis (*Trypanosoma brucei*).

By B. S. PARKIN, B.A., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

On account of the excellent results obtained by the writer with Antimosan in the treatment of bovine trypanosomiasis caused by *Trypanosoma congolense*, it was decided to test out this antimony compound against equine trypanosomiasis caused by *Trypanosoma brucei*.

The literature available does not provide any information which might lead one to expect good results from the use of antimony compounds in *T. brucei* infection. Antimony potassium tartrate has been given numerous trials in *T. brucei* infection of equines and bovines. Its use was discontinued in both of these animals on account of the poor results obtained. Naganol is, at present, the drug in use in South Africa for the treatment of this disease in horses.

Consequently no striking results were anticipated from the employment of Antimosan in the equine disease. It was therefore decided to carry out a preliminary test of Antimosan on only a few horses. The results, rather surprisingly, in these preliminary experiments have been decidedly promising and it is proposed to continue the investigations in the near future. One point of importance in favour of the use of Antimosan, as compared with Naganol, is the ease of administration.

The Antimosan, at first utilized, was the potassium salt in a 12 per cent. solution. This solution is hypertonic and it was found that it, in most cases, produced abscessation when given subcutaneously. This was avoided to a certain extent by injecting the solution slowly with massage. Later on a 7 per cent. solution, which is practically isotonic, was used and in no case did abscesses result. No particular precautions, as far as speed of injection and massage were concerned, were taken. In one case a horse was given subcutaneously, in the same spot, 40 c.c. of a 7 per cent. solution weekly for five weeks without showing any abscess formation. The site selected for the subcutaneous injections was always on the side of the neck.

T. Brucei.—Summary of Treatment with Antimosan.

Equines.	Infected on.	Appearance of Trypanosomes.	Treatment Commenced.	Number of Doses and Dosage.	Intervals.	Reappearance of Trypanosomes.	Remarks.
19079	20.3.29	24. 3.29	17.5.29	2 doses 3 gm.	Weekly	21.6.29	Subsequently horse had a severe relapse.
19079	20.3.29	—	5.7.29	5 doses 3 gm.	Weekly	25.9.29	Doing well up to 11. .30.
19431	11.6.29	19.6.29	17.7.29	5 doses 3 gm.	Weekly	Nil	Sterilised.
19472	22.5.29	1.6.29	12.6.29	2 doses 6 gm.	23 days	—	Died 9.7.29.
18095	11.6.29	19.6.29	5.7.29	5 doses 3 gm.	Weekly	13.8.29	Doing well up to 11.1.30.
18238	11.6.29	19.6.29	5.7.29	2 doses 6 gm.	Weekly	3.8.29	Doing well up to 11.1.30.
17974	26.9.29	2.10.29	—	—	—	—	Control; died 27.11.29.
19377	16.7.29	22.7.29	—	—	—	—	Control; shot *in extremis*, 27.8.29.
18328*	12.4.29	23.4.29	—	—	—	—	Control; died 22.8.29

* Equine 18328 was a donkey.

Discussion.

As will be seen from the above table certain of the horses were given more than one course of treatment. If horses were showing marked clinical indications of the disease, the Antimosan produced an almost immediate improvement in the case even though they had received a previous course of treatment.

Horses 19079 and 19431 were permitted to reach an advanced stage of the disease before treatment was instituted. The results obtained in these two animals were remarkably good. Horse 19431 became sterilized of the trypanosomes as was shown by negative smear examination for 13 weeks, by sub-inoculation into two horses with negative results and by a negative Complement Fixation Test, while horse 19079 attained a state comparable to that of premunition obtained in *T. congolense* infection of bovines by somewhat similar methods of treatment.

All of the three controls which received no treatment died. The donkey died in 19 weeks and the two horses in 8 and 7 weeks. Only one of the treated horses died and this one received a single dose on the 21st day after infection and another on the 44th day.

The progression of *T. brucei* infection in horses is considerably more rapid than that of *T. congolense* infection in bovines when the animals are kept under stable conditions. Consequently the treatment of equines by long interval methods, as has been found to be successful in *T. congolense* infection of bovines, probably would not be indicated in *T. brucei* infection of horses.

It was found that the treatment of this disease with Antimosan in doses of 3 to 6 gm. in every case produced an improvement as could be judged by the temperature curve, the decrease of oedemas when present, the decrease of the anaemia and the disappearance of the trypanosomes. The photographs (1) and (2) are of horse 19431 before and soon after the cessation of treatment. A chart of the period before and during treatment illustrating the occurrence of the trypanosomes and the temperature curve of this horse is also included. Photograph (3) is of one of the controls.

The beneficial effect of this drug, even when it does not produce sterilization, is well illustrated by horses 19079, 18238 and 18095. Notwithstanding the persistence of infection in these horses the temperature curves after treatment never showed the marked irregularity associated with *T. brucei* infection in horses. The first named horse has been infected since 15th February, 1929 and is in just as good a condition now as it was at the commencement of the experiment notwithstanding that on two occasions it was practically *in extremis*.

The strain of *T. brucei* used in these experiments was obtained from a natural case in a donkey. The weights of the horses varied from 320 to 420 kilogrammes.

Conclusions.

(1) Treatment of *T. brucei* infection of horses resulted in the sterilization of one of the five horses treated.

(2) Of the remainder one died and three are in as good a condition, notwithstanding that they still harbour trypanosomes, as when the experiment commenced.

(3) All the controls died.

(4) The indications for the use of Antimosan for the treating of *T. brucei* infection of horses are the injection of doses of about 3 to 6 grammes per 300 to 400 Kg. at intervals of less than four weeks.

HORSE 1943.

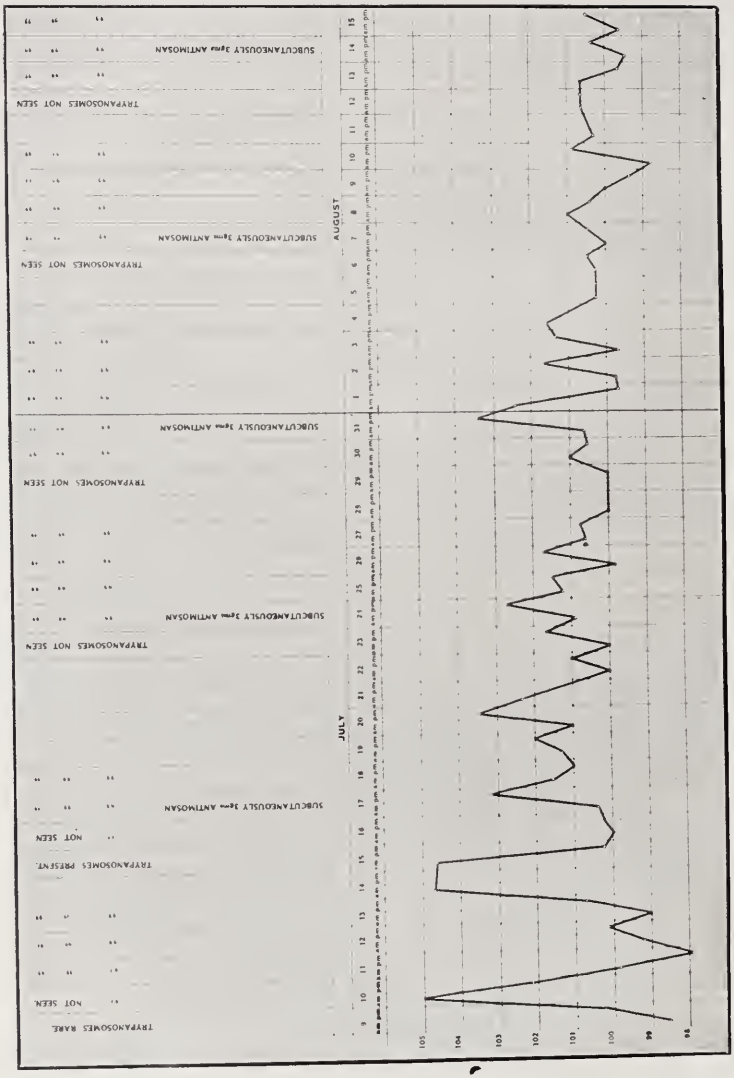

Chart 1.

Photograph 1.
H 19431. Before Treatment on 17-7-29.

Photograph 2.
H 19431. After Treatment on 30-8-29.

Photograph 3.
H 17974. Control—day of death, 27-11-29.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

A Note on the Serological Diagnosis of *Trypanosoma Congolense* Infection.

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The value of an accurate serological test for the diagnosis of any form of trypanosomiasis is undoubted in view of the difficulty frequently experienced in making a diagnosis by blood smear examination or animal subinoculations. In diseases such as dourine in horses, the problem of eradication would be insoluble without methods of serological diagnosis.

Both in the actual diagnosis of *T. congolense* infection and in the control of drug experiments with this infection, serological methods would be of great value. The adhesion reaction has been tried on a small scale but has only a limited value as it is unfortunately strain specific, which greatly restricts its application. The writer (1928) was able to show that, just as had been demonstrated by other workers with other trypanosome species, the adhesion reaction for *T. congolense* was specific and that citrated blood or serum of an animal injected with this trypanosome would not react with *T. equiperdum*, *T. lewisi* or *T. brucei*. Hornby (1929) showed that the adhesion reaction with *T. congolense* was strain specific.

The writer (1927) carried out a very limited number of complement fixation tests using a *T. congolense* antigen and showed that sera of animals with dourine did not react with it but that sera of *T. congolense* animals did. The difficulty of making a sufficient amount of antigen prevented the carrying out of more extensive testing.

As will be mentioned later in this article, the problem of producing a satisfactory antigen in the case of *T. congolense* presents certain difficulties. These have been to some extent overcome, and a large number of tests have been carried out on a herd of cattle experimentally infected with *T. congolense* at Onderstepoort. Some of these animals were undergoing drug treatment, mainly with Antimosan, and the effect of this is noticeable in the results of the tests.

In all, tests have been carried out systematically on 20 cattle, and at the time of writing are still being continued. These animals were inoculated with two different strains of *T. congolense*, some with one and some with the other. Some were inoculated with both strains at different times. One strain was obtained from Zululand by Dr. H. H. Curson of this Institute in 1922 and has been kept alive in guinea-pigs by the writer during the past 8 years. The other strain was obtained quite recently from Mr. L. E. W. Bevan, Director of Veterinary Research, Southern Rhodesia. It had recently been isolated by him from cattle with " nagana " in that country.

Technique.

Only the complement fixation test was used and the technique was essentially the same as that employed by other workers, using a rabbit anti-sheep corpuscle haemolytic system. The cattle sera were inactivated at a temperature of 60° C. for half an hour and only rarely did such sera give non-specific reactions in the tests.

Antigen production.—A satisfactory antigen for the complement fixation test in trypanosomiasis is essential. In the case of *T. equiperdum* and *T. brucei* no difficulty is experienced in making such an antigen but this is not, however, the case with *T. congolense*. In the few tests carried out by the writer (1927) only an indication was obtained as to the value of the *T. congolense* antigen. Subsequently the writer alone, and with a colleague, Mr. D. A. Lawrence, has at various times attempted to produce a satisfactory antigen from *T. congolense* but until recently, has failed. Many methods have been tried including such methods as the sugar flotation ones for worm eggs. Prolonged attempts were made to get a really heavy infection in small animals, more particularly white rats, but without success. In the case of guinea-pigs the heaviest infections occurred soon after the trypanosomes were first seen in the blood (first paroxysm) and if death did not occur then, the later appearances in the blood were with fewer parasites, and just before death they might even be rare. It should be mentioned that these attempts were made with a strain from Zululand which has by now been kept going in guinea-pigs for eight years. It has not fallen off in virulence for these animals but is no longer very virulent for cattle.

More recently a strain from Southern Rhodesia has been obtained which is more pathogenic for small animals and cattle than the other strain and it is with this strain that most of the work to be described has been done. This Rhodesian strain gives heavier infections in guinea-pigs than the other one does, but under no conditions has an infection such as can be obtained with *T. equiperdum* or *T. brucei* been seen.

The opportunity for doing experimental work with *T. congolense* on a big scale having occurred in connection with chemotherapy in its treatment, another attempt was made to produce a satisfactory antigen, this time with more success. At first antigens made of extracts of spleen, liver and heart muscle of infected guinea-pigs were tried but the results with them were unsatisfactory. It was observed that, even with a heavy infection of *T. congolense* in a guinea-pig or white rat, no success usually attended centrifugalizing out of the trypanosomes as done in making *T. equiperdum* antigen as described by Watson (1920) and carried out by the writer (1927). The reason for this was found to be probably connected with the attachment of most of the parasites to red corpuscles or leucocytes, a feature described by van Saceghem (1922) and familiar to all workers with *T. congolense*.

After centrifugalization a thin layer of trypanosomes might be found at the surface of the blood but numerous parasites at any depth as well. Agglutination of the parasites by a foreign serum was not found satisfactory as a means of getting them out, the clumps

remaining at all levels after centrifugalization. The idea of detaching the trypanosomes by killing them was next tried and it was found that this did actually cause detachment. Of the chemicals used for this purpose, formalin has proved the most satisfactory and in a dilution of 1 part to 1,000 parts of blood has given the best results. Very occasionally formalin with guinea-pig blood has produced a viscidity of the blood which rendered centrifugalization impossible.

The procedure in making the antigen is essentially the same as with *T. equiperdum*. The guinea-pigs or rats are bled to death into 1 per cent. sodium citrate in normal saline, using equal parts of blood and citrate of saline. The formalin is added and within a few minutes the trypanosomes are all dead. Centrifugalization is done in fine tubes with a diameter of .25 of an inch and usually good trypanosome layers are obtained. These layers are added together in one or two tubes and normal saline is added to make a 50 per cent. suspension of trypanosomes in the tubes. Re-centrifugalization is then done. This results in thick layers of trypanosomes with a few red corpuscles at the bottom. The trypanosome layers are then all added together and preservative is added in the proportion of 2 parts to 1 of the parasites. The preservative used is that recommended by Watson (1920) and consists of a 10 per cent. glycerine and normal saline mixture plus .1 per cent. of formalin. The antigen is preserved in small pipettes each holding about .2 to .5 c.c., about sufficient for a test on 15 to 20 animals. Slides made from this antigen have shown that it contains trypanosomes in enormous numbers with practically no red corpuscles or leucocytes.

The table gives the histories of the animals in the drug treatment experiments and a comparison with the reactions to the complement fixation test during a period of four months. The tests and drug treatment are still being continued and it is not the object of this paper to do more than show that there is some relationship between the reactions and the state of infection of the animals.

One may briefly consider the animals mentioned in the table as regards their history in the drug treatment experiments comparing their state of infection with their reactions to the complement fixation test.

Bovine 416.—This animal continued to react strongly to the serum test after it ceased to show trypanosomes in the blood. This may apparently happen in some cases and there is always the possibility that the parasites were not completely got rid of.

Bovine 2464.—The history in this case is similar to that of 416. The apparent sterilization did not however take place until the middle of January, 1930.

Bovine 2468.—This animal was constantly infected and the reactions correspond very well with the infection.

Bovine 2471.—In this case there was a short negative phase in the serological reactions. This may have been associated with the treatment.

Borine 2473.—The infection was practically constant all the time in this case and the serological reactions correspond.

Bovine 2702.—The state of infection and the serological tests correspond fairly well.

Borine 2709.—The serological reactions were strong throughout. This animal was premune, that is it remained apparently well in spite of the presence of trypanosomes in the blood, and could not be reinfected.

Bovine 2714.—The reaction to serological test continued to be strong for at least a month after trypanosomes could no longer be demonstrated by blood smear examination.

Borine 2727.—This animal had a negative phase in its serological reactions but again became positive when reinfected.

Bovine 2994.—The negative phase here corresponds to the disappearance of the trypanosomes.

Borine 3502.—As in 2994.

Borine 3506.—The serological reaction has remained positive or partially positive after the disappearance of trypanosomes.

Borine 3517.—An animal accidentally infected, a possible mechanical transmission. Only tested once so far.

Borine 3518.—An uninfected animal used as a negative control.

Borine 3520.—This animal has been practically constantly infected and has been positive to the serological test whenever it was done.

Borine 3522.—As in 3518.

Borine 3524.—In this case the negative phase corresponded to the disappearance of trypanosomes.

Borine 3525.—This animal reacted to the serological test while showing trypanosomes.

Borine 3527.—In this case the state of infection and the reactions to the serological test agree very well.

Borine 3627.—As in 3527.

Borine 3636.—The state of infection and the serological reactions agree fairly well.

Comments.

In the tests done with *T. congolense* sera it has not been found that the same clear cut reactions have been obtained as one gets with *T. equiperdum* antigen and dourine sera. It has been found that one has to put a time limit on the reaction and read it at certain intervals. For instance it was found that sera might give definite positive reactions after half an hour at 37° C. but might partially haemolyse if left for an hour. This may mean that the combination of antigen

and antibody is not strong and apparently *T. congolense* does not cause the production of antibodies to the extent that *T. equiperdum* or *T. brucei* does. The actual value of the complement fixation test for " Nagana " (*T. congolense* infection) in the field has not yet been determined but there is every probability that it may prove useful, especially in those cases where one is unable to find the trypanosomes, in blood smears. On account of mixed infection it would be necessary as a rule to test the sera with a *T. brucei* antigen as well, and one could even substitute *T. equiperdum* antigen for the *T. brucei* one if the necessity for doing so arose.

Conclusions.

(1) The complement fixation test has been used in the diagnosis of *T. congolense* infection.

(2) Its value in the case of the experimental disease has been demonstrated and it has been shown that it may have a use as an indicator of the success or failure of drug treatment.

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Complement Fixation Tests in *T. Congolense* Infection in Cattle.

The history of the infection in each animal is given over its particular tests. The sera were tested in amounts of .2 and .1 c.c. respectively.

No.	28.11.29.		19.12.29.		4.1.30.		9.1.39.		16.1.30.		7.2.30.		13.2.30.		20.2.30.		6.3.30.		20.3.30.	
	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1	·2	·1
416.....	Infected with Zululand strain in April, 1929. Reinfected in September, 1929. Treated in October and apparently sterilized.																			
	PH	PH	H	H	PH	PH	PH	PH	ACH	ACH	PH	PH	CF	CF	CF	CF	CF	CF	—	—
2464.....	As 416. Reinfected with Rhodesian strain in September, 1929. Treated in October and showed tryps. until 13th January, 1930.																			
	CF	CF	PH	ACH	PH	PH	ACF	PH	ACF	ACF	ACF	PH	CF	CF	CF	CF	PH	PH	ACF	ACF
2468.....	Infected with Zululand strain, April, 1929. Treated. Reinfected September, 1929, with Rhodesian strain. Still infected 20th March, 1930.																			
	—	—	PH	PH	ACF	ACF	ACF	ACF	ACF	PH	ACF	ACF	—	—	ACF	ACF	CF	ACF	—	—
2471.....	Infected April, 1929, with Zululand strain. Treated. Reinfected September, 1929. Still infected on 20th March, 1930.																			
	PH	PH	—	—	PH	PH	H	H	H	H	CF	CF	CF	CF	CF	ACF	CF	CF	CF	PH
2473.....	Infected April, 1929, with Zululand strain. Treated. Reinfected September, 1929. Still infected on 20th March, 1930.																			
	—	—	—	—	—	—	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF
2702.....	Infected 10th January, 1929, Zululand strain. Treated. Reinfected September, 1929. Treated. Still infected 20th March, 1930.																			
	CF	CF	—	—	CF	CF	CF	CF	PH	PH	CF	CF	PH	PH	ACF	ACF	—	—	—	—
2709.....	Infected 10th January, 1029, Zululand strain. Became premune. Reinfection unsuccessful. Treated, but still infected 20th March, 1930.																			
	—	—	—	—	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF
2714.....	Infected September, 1929, with Rhodesian strain. Treated in February, 1930. No tryps. seen since 20th February, 1930.																			
	—	—	—	—	ACF	PH	ACF	PH	PH	PH	ACF	PH	ACF	ACF	CF	CF	ACF	ACF	ACF	ACF
2727.....	Infected June, 1929, with Zululand strain. Reinfected 17th January, 1930, with Rhodesian strain. Treated, but still infected 20th March, 1930.																			
	—	—	—	—	—	—	H	H	H	H	ACF	ACF	PH	PH	PH	PH	CF	CF	—	—
2994.....	Infected with Rhodesian strain. No trypanosomes seen since 13th January, 1930. Not treated.																			
	CF	CF	CF	CF	CF	CF	CF	CF	CF	CF	PH	PH	H	H	PH	PH	H	H	H	H
3502.....	Infected June, 1929, Zululand strain. Treated from July to November, 1929. Sterile since January, 1930.																			
	PH	PH	—	—	—	—	PH	ACH	ACH	ACH	PH	PH	PH	PH	ACH	ACH	H	H	—	—

3506.....	Infected June, 1929, Zululand strain. Treated. Reinfected September, 1929. Rhodesian strain. Treated. Sterile.																			—
	CF	CF	PH	PH	PH	PH	ACF	ACF	PH	PH	H	H	CF	PH	PH	PH	PH	PH	PH	PH
3517.....	Accidentally infected in February, 1930. Not treated.																			
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	CF	CF
3518.....	Uninfected. Used as a negative control.																			
	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
3520.....	Infected April, 1929, with Zululand strain. Treated. Reinfected. Reinfected September, 1929. Still infected on 20th March, 1930.																			
	CF	CF	—	—	CF	CF	—	—	—	—	—	—	—	—	PH	ACF	CF	CF	—	—
3522.....	Uninfected. Used as a negative control.																			
	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
3524.....	Infected June, 1929, Zululand strain and reinfected September, 1929. Treated 16th October, 1929. Sterile since November, 1929.																			
	—	—	—	—	ACH	ACH	H	H	H	H	H	H	CF (non-specific)		H	H	H	H	PH	PH
3525.....	Infected September, 1929. Sterile since January, 1930.																			
	CF	CF	CF	CF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3527.....	Infected with Zululand strain April, 1929. Reinfected September, 1929. Treated 20th November, 1929. No tryps. seen since January, 1930.																			
	CF	CF	CF	CF	PH	PH	PH	PH	PH	PH	H	H	H	H	H	H	H	H	H	H
3627.....	Infected with Zululand strain April, 1929. Reinfected September, 1929. Treated 20th November, 1929. No tryps. seen since January, 1930.																			
	CF	CF	ACF	PH	ACF	ACF	PH	PH	PH	PH	ACF	ACF	PH	PH	PH	PH	ACH	ACH	ACH	ACH
3636.....	Infected with Rhodesian strain. Not treated. Still infected on 20th March, 1930.																			
	—	—	—	—	CF	CF	CF	ACF	ACF	ACF	PH	ACH	CF	PH	ACF	ACF	PH	PH	CF	ACF

CF = complete fixation.
ACF = almost complete fixation.
PH = partial haemolysis.
ACH = almost complete haemolysis.
H = haemolysis.

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A Short Note on the Spirochaetal Wound Infection of Pigs.

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Spirochaetal wound infection represents a chronic disease of swine produced by the *Spirochaeta suilla* or the associated fusiform bacillus or both in the course of which inflammatory and necrotic processes take place in connection with the skin and subcutaneous tissues, bringing about abscessation and induration. In the minority of cases lesions are found in organs e.g. the lungs, and urinary bladder.

History.

The occurrence of spirochaetes in pigs was first mentioned by Smith (1899), who found fine spirochaetes in the submucous cysts of the large intestine of the pig. (Dodd 1906) described spirochaetes in the discharge of superficial ulcers of the skin in pigs that were sent to the Government Veterinary Bacteriological Laboratory at Pretoria.

In West Australia, Cleland (1908) found spirochaetes in castration wounds of pigs. Gilruth (1910) found spirochaetes in ulcerating wounds on various parts of the body, the head, knee, scrotum and in the submucous cysts of the large intestines. Gill (1929) describes a spirochaetal wound infection in pigs in New Zealand. Knuth and Du Toit give numerous references on the occurrence of spirochaetes in pigs.

In South Africa spirochaetosis, as it is called, occurs quite frequently and is fairly widespread. Cases have been recorded in or near Vryburg, Pietermaritzburg, Kimberley, Vereeniging, Indwe, Tweespruit, Excelsior near Bloemfontein, and Radium in the Northern Transvaal.

Aetiology.

In every case that was submitted to treatment *Sp. suilla* and a fusiform bacillus were found. In those cases which had open lesions various other bacteria were found in addition. The question naturally arises whether the spirochaetes or the fusiform bacilli or both are responsible for this condition.

The spirochaetes are motile, measure about 3-16μ and consist of from 2-6 spirals. They are easily stained by the ordinary stains used for blood smears. The stains used in the routine examination

carried out regularly for control of the treatment was May-Grünewald—Giesma. During the course of experimental treatment numerous blood smears were examined from the treated and untreated pigs but in no case were spirochaetes found.

Epizootology.

Only occasionally do a number of cases occur at the same time although the total number throughout a year might represent quite a serious economic loss. Probably more cases are noted during the rainy season. This is likely due to the fact that the conditions under which pigs live at such a time become worse. There seems to be no doubt that the incidence of the disease must be, to a great extent, ascribed to bad hygiene. Especially is this noted when there is associated with bad hygiene, overcrowding, and broken concrete floors all of which predispose to wounds and subsequent infection. Operative wounds, especially as a result of castration, associated with dirty muddy styes result commonly in scrotal infections. Gill laid great emphasis on the fact that pigs which were kept under insanitary conditions were the ones most commonly affected with spirochaetosis.

Susceptibility.

Pigs of all breeds, of both sexes and of any age may be affected. Most frequently the condition is found in young pigs; this is probably due to the fact that scrotal lesions following on castration, formed the majority of lesions noted. Pigs castrated at the age of eight to twelve weeks were as susceptible as those castrated at the age of two to three weeks.

Anatomical Changes.

The micro-organisms apparently enter through abrasions and wounds into the skin and subcutaneous tissues. The lesions produced vary somewhat with the location. The most common lesion is the scrotal and is due to the infection of the castration wound. These scrotal lesions (See figures No. 9 and 11) become extremely large coming, at times, into contact with the ground (Canham) when the pig is standing up. They consist of fibrous tissue in the interspaces of which one finds a collection of a grey to a greyish white evil smelling pus. The skin of the scrotum may give way permitting of the escape of some of the pus. Open wounds discharging pus containing necrotic material then persist. The foot cases usually do not show soft centres, and are at times so indurated that it is practically impossible to insert a needle into the lesion. In some cases a limb may be increased from the hock downwards several times the normal size. The majority of leg lesions are however of the type as illustrated in figures Nos. 7 and 8. The superficial skin lesions are in the form of ulcers covered with pus and having borders of ragged outline. The shape of these is elliptical or circular. The skin gradually sloughs away at the periphery and there is destruction of the underlying tissues. One pig (figure No. 1) showed lesions in the form of large fluctuating swellings beneath the skin. The material aspirated from these lesions did not show spirochaetes on microscopic examination but after commencement of the treatment spirochaetes were

found in the pus discharging from the injection puncture. In this case there is the possibility that infection set in subsequent to commencement of treatment.

A number of pigs shewed, in addition to the various spirochaetal lesions, a broncho-pneumonia in the lesions of which spirochaetes could be demonstrated in four cases out of fourteen. In one case a spirochaetal abscess about 1 cm. in diameter was found in the wall of the urinary bladder. Enteritis when present could be ascribed to the presence of *Ascaris suum*.

Symptoms.

The symptoms noted in the pigs collected for experimental treatment were probably mostly due to the ascaris infection which was present at the same time. The presence of even extremely large lesions did not apparently produce inappetence. The temperature however showed marked fluctuations varying from 99° F. to 105° F. but it was quite noticeable that such fluctuations were absent in those pigs that had recovered from spirochaetosis as the result of treatment. In a number of cases symptoms of pneumonia were present and it was in these particularly that loss of condition was noted. In those that were not severely infected with ascaris and which did not suffer from pneumonia the condition was fair. Pigs that had foot lesions were lame. The mortality from spirochaetosis itself is not high.

Diagnosis.

The diagnosis is made by the examination of the pus with dark field illumination or in stained smears.

Treatment.

Many experiments have been carried out to obtain a suitable treatment for this disease but without any satisfactory results. The usual method that has been employed was the intravenous injection of drugs chiefly arsenicals. At Onderstepoort this method of administration had been given a good trial. The drugs used were neosalvarsan, Bayer 205., liquor arsenicalis and arsenious oxide. The results were unsatisfactory. Of the nineteen pigs in the experiment fifteen were treated and four were kept as controls. Of the controls one died, one showed slight improvement, and two recovered. Of the fifteen pigs that were treated only five recovered . The treatment was persisted in to such an extent that two died from arsenical poisoning.

It was decided when further cases were received that, since the intravenous method of administration did not apparently hold out much hope of success, to try the administration of various drugs directly into the lesions. Parkin of this Institution thereupon commenced treating the pigs by either the injection with a hypodermic syringe and needle of the selected drug directly into the lesion or the application of a solution of it on to the surface of those which were of an ulcerative type. He treated one pig by means of the intravenous injection of neosalvarsan. These experiments and their continuation by the first named author are recorded in this article.

The table gives the details of the experiment. In all sixteen pigs were used, five of these being kept as controls. None of the controls recovered. A certain amount of selection of cases was necessary as it was soon discovered that the foot and leg lesions did not lend themselves to the methods employed on account of the difficulty experienced in introducing the needle into the extremely hard lesions.

TABLE.

Porcine Spirochaetosis—Experimental Treatment.

No. of Pig.	Lesion.	Drug.	Each Dose.	No. of Doses.	Method of Administration.	Remarks.
696	Shoulder	C.D.D.P.*....	0·14 gm...	4	Into lesion...	Complete recovery.
	Axilla....	,, ...	0·14 gm...	10	,, ...	,, ,,
710	Serotum.	,, ...	0·07 gm...	4	,, ...	,, ,,
712	Shoulder	,, ...	1·4% sol..	3	On lesion....	,, ,,
700	Serotum.	Atoxyl.....	0·01 gm...	1	Into lesion...	,, ,,
		,, ...	0·02 gm...	3	,, ...	—
		,, ...	0·5 gm.....	1	,, ...	—
		,, ...	1·0 gm....	11	,, ...	—
707	Elbow...	,, ...	0·01 gm...	3	,, ...	Complete recovery.
705	Leg.....	,, ...	0·04 gm...	1	,, ...	—
		,, ...	0·1 gm....	1	,, ...	—
		,, ...	0·2 gm....	2	,, ...	No improvement.
		C.D.D.P.....	1·4% sol..	12	On lesion....	,, ,,
706	Serotum.	Antimosan...	0·72 gm...	1	Into lesion...	—
		,, ...	1·44 gm...	2	,, ...	No improvement.
		Atoxyl.....	0·05 gm...	1	,, ...	—
		,, ...	0·1 gm....	1	,, ...	—
		,, ...	0·15 gm...	2	,, ...	—
		,, ...	0·2 gm....	3	,, ...	—
		,, ...	0·5 gm....	5	,, ...	—
		,, ...	1·0 gm....	9	,, ...	Slight improvement.
698	Leg.....	Neosalvarsan.	0·37 gm...	1	Intravenous..	No improvement. Died from broncho-pneumonia.
		,, ...	0·75 gm...	2	,, ...	—
704	Serotum.	,, ...	0·37 gm...	1	Into lesion...	Complete recovery.
		,, ...	0·75 gm...	5	,, ...	—
697	Serotum.	Arieyl.....	6 c.c.....	1	,, ...	No improvement. Died from pneumonia and peritonitis.
		,, ...	10 c.c.....	6	,, ...	—
703	Shoulder	,, ...	5 c.c.....	1	,, ...	Slight improvement. Died from spirochaetal broncho-pneumonia.
		,, ...	6 c.c.....	1	,, ...	—
		,, ...	10 c.c.....	4	,, ...	—
		,, ...	20 c.c.....	1	,, ...	—

* C.D.D.P.—Cooper's Double Dipping Powder.

Discussion of the Experimental Treatment.

In all twelve lesions were treated two of which were in Pig No. 696. The treatment was carried out at weekly intervals. Complete recoveries were obtained in seven lesions, slight improvement in two, one of which had previously been treated with Antimosan, and no improvement in four.

The intravenous injection of Neosalvarsan produced no improvement but the injection of this drug into a well-marked scrotal lesion produced a very rapid and striking complete recovery (figures Nos. 9 and 10). Similar good results were obtained by the use of Cooper's Double Dipping Powder in a 1.4 per cent. solution administered directly into the lesion. Figures Nos. 1 and 2 illustrate the results obtained. In pigs 712 and 705 the application of the solution of Cooper's Double Dipping Powder was carried out by applying a piece of cotton wool soaked with the solution to the lesions for a period of two and ten minutes respectively. In the former case the lesion was similar to that of pig 703 (figure No. 5) being of the nature of a large superficial ulcer, whereas in the latter the lesion was a hard, fibrous one with only a small portion of the skin sloughed away. The difference of the results obtained in these two cases is undoubtedly due to the difference of the type of lesions. The results obtained with Aricyl and Atoxyl were not very satisfactory. In the latter drug the actual amount of arsenic introduced should have been sufficiently high, judging by the amount present in the more successful drugs, to produce good results. The amount of arsenic in Aricyl however was on the low side being only 0.003 gm. in each 5 c.c. Antimosan was not given a full trial.

Only two leg cases were treated. Good results in the treatment of these were not anticipated on account of the already mentioned difficulty in connection with the introduction of the drugs. Four untreated pigs showed no improvement during the time covered by these experiments. One control that was considered as a recovery was found to be still infected with spirochaetes when the apparently healed lesion of the nasal region was examined at post-mortem.

Conclusion.

1. Remarkably successful results are reported in the treatment of a few cases of spirochaetosis in pigs by the use of arsenical preparations either injected into or applied on to the lesion.

2. Good results were obtained with Cooper's Double Dipping Powder in four cases, with Neosalvarsan in one and with Atoxyl in another.

3. The intravenous injection of various arsenicals proved to be inefficacious.

Prevention.

A great deal can undoubtedly be done to reduce the incidence of the disease by means of the introduction of adequate sanitary conditions. At Tweespruit it was noticed that comparatively few cases occurred when dipping with arsenicals was regularly practised. The isolation of affected pigs is advisable. Pigs should receive a suitable diet and be kept as free as possible from worm infestation in order to maintain the resistance of the animals.

Immunity.

Apparently there is no immunity produced by the result of an infection for recurrence of the condition has been observed.

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FIG. 1.
Pig 696. Before Treatment.

FIG. 2.
Pig 696. After Treatment.

FIG. 3.
Pig 707. Before Treatment.

FIG. 4.
Pig 707. After Treatment.

FIG. 5.
Pig 703. Ulcerative type of lesion.

FIG. 6.
Pig 712. Result of treatment of ulcerative lesion similar to that of pig 703.

FIG. 7.
Pig 698. Leg Lesion.

FIG. 8.
Pig. 702. Leg Lesion.

FIG. 9.
Pig 704. Before Treatment.

FIG. 10.
Pig 704. After Treatment.

Fig. 11.
Pig 706. Before Treatment.

Fig. 12.
Pig 706. After Treatment.

Section II.

Virus Diseases.

P. J. du Toit and R. A. Alexander — The Immunization of Horses against Horsesickness by the Use of Formalized Virus.

The Immunization of Horses against Horse-sickness by the Use of Formalized Virus.

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As a result of the publication of the excellent results obtained from the use of formalized spleen virus in the immunization of dogs against Distemper by Laidlow and Dunkin (1928), the possibility of applying the same principle to the immunization of horses against Horsesickness was considered immediately.

The work was commenced early in January, 1929, and this article should be regarded as merely serving the purpose of placing on record the results obtained up to the present with this method. As it is being published only in the form of a progress report on research which is far from complete, no attempt is made to discuss the relative merits of previous methods of immunization and no criticism is offered of the reported results obtained by the use of a similar method by other authors. This discussion will form an integral part of a comprehensive article to be published on the whole subject of the immunization of equines against Horsesickness at a future date.

Technique.—As the original technique has remained unaltered since the commencement of the work, except in certain minor points which will be indicated from time to time, a short description will prevent any confusion in following the various experiments outlined below.

To obtain infective material susceptible horses are injected intrajugularly with 5 c.c. of O. virus, the most virulent virus encountered during the previous work carried out on Horsesickness at Onderstepoort. This O. virus in turn consists of blood drawn from the jugular vein of an animal at the height of the Horsesickness reaction into a preservative anticoagulant mixture of 5 gm. Potassium oxalate, 5 c.c. Phenol, 500 c.c. Glycerine and 500 c.c. water. After injection with this virus the temperature rises rapidly to 106-108° after an incubation period of approximately 3 days, remains elevated for a variable length of time, usually 1-3 days, and death occurs by crisis. The temperature chart of a case of Horsesickness caused by O. virus is characterized by its remarkable constancy, as indicated by experience gained from many hundreds of cases (cf. Figure 1).

FIG. 1

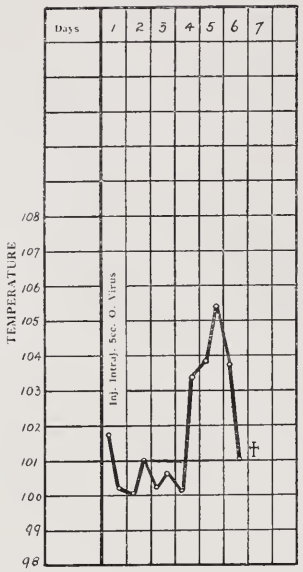
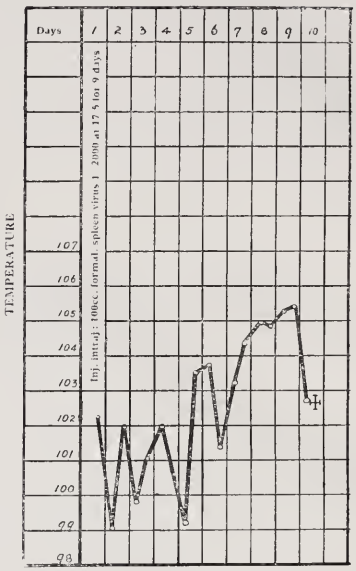


FIG. 2. HORSE 19382.



To obtain virulent blood for formalization, a suitable quantity is withdrawn from the jugular vein when the animal is *in extremis*, and either defibrinated by shaking with glass beads, or prevented from clotting by mixing with 12 per cent. sodium citrate solution in the proportion of 1 part citrate solution to 10 parts of whole blood.

When a tissue emulsion is required the animal is shot *in extremis* and the required organs immediately removed with all the speed and necessary precautions to ensure absence of bacterial contamination from without. The organs are then passed through a sterile Latapie mincer into weighed 2-litre Erlenmeyer flasks plugged with non-absorbent cotton wool plugs through which pass a glass tube 8 mm. in diameter suitably stoppered and used for the subsequent addition of sterile saline and formalin. By difference the weight of the tissue pulp is obtained and to this is added 4 times that volume of sterile .9 per cent. saline solution. This produces an approximately 20 per cent. emulsion of organ tissue in saline, sufficiently accurately determined for all practical purposes since the original mathematical error remains constant throughout. To this emulsion formaldehyde is added in the form of a 4 per cent. solution until the required concentration is obtained in the mixture.

Before injection, this formalized emulsion is filtered through a double thickness of sterile butter muslin to remove the coarsest particles.

Immunity tests.—The immunity of horses to Horsesickness is tested by the intrajugular injection of 5 c.c. O. virus, a dose which can be relied upon to produce the reaction shown in Figure I in a susceptible animal.

EXPERIMENT 1. (S. 3784.)

Object of the Experiment: To determine whether a concentration of 1 part of formaldehyde to 1,000 parts of an emulsion of spleen or liver will be sufficient to render the injection of such material safe, and whether horses which have received such an injection will be immune against Horsesickness.

Method.—The spleen and liver were removed from a horse No. 19254, which had been injected with O. virus and which was killed *in extremis*. Formalin was added to a 20 per cent. emulsion of this spleen and liver until the final concentration was 1 part of formaldehyde to 1,000 parts of emulsion. Each formalized tissue emulsion was divided into 2 parts, the one being kept for 48 hours at 24° C., the other for 48 hours at 37° C. before injection. Four horses were used in the experiment and the injections were made subcutaneously in 10 c.c. amounts with the following results:—

Horse No.	Organ.	Formaldehyde Concentration.	Time and Temp.	Result.	Immunity Test.	
					Interval.	Result.
19263	Liver.....	1 : 1,000	48 hours at 37° C.	No reaction	15 days	Died.
19357	Liver.....	1 : 1,000	48 hours at 24° C.	Thermal reaction	21 days	Died.
19353	Spleen.....	1 : 1,000	48 hours at 24° C.	Died. Nuttaliosis	—	—
19355	Spleen.....	1 : 1,000	48 hours at 37° C.	No reaction	15 days	Died.

Conclusion.—A concentration of 1:1,000 formaldehyde when allowed to act on infective spleen or liver emulsion for 48 hours at 37° C. completely inactivates the virus, but one injection of 10 c.c. of this inactivated virus produces no immunity against the subsequent intravenous injection of 5 c.c. of O. virus 15 days later. On the other hand, when a similar concentration of formaldehyde is allowed to act for 48 hours at 24° C. the virus is not completely inactivated, but no trace of any immunity can be established.

Discussion.—The reason why Horse No. 19353 should have succumbed to Nuttaliosis is not quite clear, and unfortunately suitable diagnostic subinoculations were not carried out at the time. However, the subsequent experiments have made it seem probable that the relapse to Nuttaliosis was due to an intercurrent attack of Horsesickness characterized by an unusually long incubation period, this period being prolonged owing to the partial inactivation of the virus.

As the results obtained from this experiment indicate that the virus is completely inactivated by a concentration of 1:1,000 formaldehyde at 37° C., it was considered advisable to ascertain the effect of formaldehyde at lower concentrations. The immediate object was to determine the lowest concentration which would influence the virus so that subsequent experimentation could be undertaken to determine the optimum concentration at which the virus would be sufficiently attenuated to be safe and would at the same time confer an adequate immunity.

EXPERIMENT 2. (S. 3810.)

Object of the Experiment: to determine whether a concentration of 1 part of formaldehyde to 10,000 parts of blood, and of an emulsion of equal parts of spleen and liver tissue would be sufficient to render the injection of such material safe, and whether horses which had received such an injection would be immune against Horsesickness.

Method.—Virulent defibrinated blood, and spleen and liver were obtained from Horse No. 19357 which was killed *in extremis* after previous infection with O. virus. Formalin was added to the defibrinated blood and to a 20 per cent. emulsion of equal parts of the spleen and liver tissue until the final concentration was 1 part of formaldehyde to 10,000 parts of blood or tissue emulsion. As before, each was divided into 2 parts, the one being left for 48 hours at 37° C., the other for 48 hours at 24° C. before injection. Injections were made subcutaneously in 10 c.c. amounts with the following results:—

Horse No.	Organ.	Formaldehyde Concentration.	Time and Temp.	Result.	Immunity Test.	
					Interval.	Result.
19372	Blood.....	1 : 10,000	48 hours at 24° C.	No reaction	15 days	No reaction.
19375	Blood.....	1 : 10,000	48 hours at 37° C.	Died.....	—	—
19376	Spleen and blood...	1 : 10,000	48 hours at 37° C.	Died.....	—	—
19374	Spleen and blood...	1 : 10,000	48 hours at 24° C.	Died.....	—	—

Conclusions.—A concentration of 1 part of formaldehyde to 10,000 parts of defibrinated blood or a mixture of equal parts of spleen and liver tissue is not sufficient to inactivate the contained virus; in fact, it would appear that when allowed to act for 48 hours at either 37° C. or 24° C. it has no appreciable influence upon the virulence of the virus.

Discussion.—The reason why Horse No. 19372 failed to react to the formalized virus and also gave no reaction in the immunity test is obviously due to the presence of a previous strong immunity. Salted animals of this nature are occasionally encountered (less than 1 per cent.), but as they are comparatively rare they cannot be considered to have any serious effect upon the conclusions drawn from the results of experiments.

From this experiment it is apparent that the minimum concentration of formaldehyde at which inactivation of the virus takes place lies somewhere between the wide limits of 1:1,000 and 1:10,000.

As no immunity is produced by the subcutaneous injection of 10 c.c. of inactivated virus it was considered advisable to ascertain the effect of much larger doses of completely and partially inactivated virus.

EXPERIMENT 3. (S. 3854.)

Object of the experiment: to determine whether a concentration of 1 part of formaldehyde to 5,000 parts of defibrinated blood will

inactivate the virus and at the same time produce a solid immunity when injected into susceptible horses.

Method.—Blood was withdrawn from the jugular vein of a virus horse at the height of the reaction to O. virus (when it was being used as a donor for the hyperimmunization of immune horses) and defibrinated by shaking with beads. Formalin was then added until the concentration was 1 part of formaldehyde to 5,000 parts of defibrinated blood, and stored at room temperature (20° C.) for 48 hours before being injected intrajugularly into 2 susceptible horses, with the following results:—

Horse No.	Material.	Amount Injected.	Formaldehyde Concentration.	Time and Temperature.	Result.
19433	Blood.....	10 c.c.	1 : 5,000	48 hours at 20° C.....	Died.
19437	Blood.....	300 c.c.	1 : 5,000	48 hours at 20° C.....	Died.

Conclusion.—A concentration of 1 part of formaldehyde to 5,000 parts of defibrinated blood when stored at 20° C. for 48 hours does not inactivate the virus.

Discussion.—The result obtained from this experiment indicates that the concentration of formaldehyde just sufficient to inactivate the virus lies somewhere between 1:1,000 and 1:5,000. As the limit of safety has now been narrowed down considerably, it was decided to ascertain the effect on the virus of varying the temperature at which the formaldehyde is allowed to act.

EXPERIMENT 4. (S. 3862.)

Object: to determine the effect of varying concentrations of formaldehyde on the O. virus content of virulent defibrinated blood, the formaldehyde being allowed to remain in contact for varying lengths of time at different temperatures.

Method.—Blood was drawn from the jugular vein of a virus horse *in extremis* during the horsesickness reaction following injection with O. virus and immediately defibrinated by shaking with beads. This material was then divided into three portions and treated as follows:—

(*a*) Formalin added to make a final concentration of 1 part of formaldehyde to 1,000 parts of blood, and placed in cold storage at 3° C. Two 100 c.c. subcutaneous injections of this material were given to one horse No. 19472 after 48 hours and 9 days respectively.

(*b*) Formalin added to make a final concentration of 1:2,000 and placed in a cool chamber at 17.5° C. One horse No. 19381 was injected subcutaneously with 100 c.c. after 48 hours and one horse No. 19382 after 9 days.

(*c*) Formalin added to make a final concentration of 1:5,000 and kept at room temperature at 20° C. One horse No. 19490 received 100 c.c. subcutaneously after 9 days.

Horse No.	Material.	Dose.	No. of Injections.	Formaldehyde Concentration.	Temp. and Time.	Reaction.	Immunity Test.	
							Interval.	Result.
19472	Blood..	100 c.c.	2	1 : 1,000	3° C after 48 hours and 9 days	Temp. and Dikkop	22 days	No reaction.
19381	Blood..	100 c.c.	1	1 : 2,000	17·5° C. for 48 hours	Died after delayed reaction		
19382	Blood..	100 c.c.	1	1 : 2,000	17·5° C. for 9 days	Died after delayed reaction		
19490	Blood..	100 c.c.	1	1 : 5,000	20° C. for 9 days	Died.		

Conclusions.—A formaldehyde concentration of 1:5,000 after contact with O. virus for 9 days at 20° has no appreciable effect upon the virulence.

A concentration of 1:2,000 acting for 48 hours at 17.5° C. has some effect on the virus as indicated by the death of the recipient after a slightly prolonged incubation period, but when allowed to remain in contact for 9 days partially inactivates the virus as indicated by an incubation period prolonged by 2 days and death after 10 days. (See fig. 2.)

A concentration of 1:1,000 incompletely inactivates the virus after 48 hours at 3° C., as indicated by recovery following a fever reaction with clinical symptoms of horsesickness after an incubation period lengthened to 8 days. The second injection which was made 1 day before the initial rise in temperature had no apparent effect upon the course of the reaction. After 22 days a solid immunity against horsesickness was produced.

Discussion.—Any suggestion that this horse No. 19472 was immune to horsesickness at the commencement of the experiment cannot be entertained, since it showed a normal mild horsesickness reaction with definite clinical symptoms from which it made an uninterrupted recovery. Blood taken at the height of the febrile reaction and subinoculated into a susceptible horse No. 19435 proved to possess a virulence equal to that of the original O. virus.

The decidedly encouraging result obtained with the one horse in this experiment led to the idea of attempting immunization by the subcutaneous injection of virus inactivated by formaldehyde concentrations of approximately 1:1,000, the inactivation being allowed to occur during storage at 3° C. for 10 days.

EXPERIMENT 5. (S. 3862 A.)

Object: to determine the effect of concentrations of formaldehyde of approximately 0.1 per cent. on the O. virus contained in defibrinated blood after storage at 3° C. for 10 days, and, if inactivated, to ascertain the immunizing value of such material.

Method.—Formalin was added to virulent defibrinated blood obtained as before to give the following concentrations of formaldehyde:—

a. 1:500.
b. 1:750.
c. 1:1,000.
d. 1:1,500.

The formalized material was placed in cold storage at 3° C. for 10 days and then injected in 100 c.c. amounts either subcutaneously or intrajugularly, as indicated, with the following results:—

Horse No.	Material.	Dose.	No. of Injections.	Method.	Formaldehyde Concentrations.	Reaction.	Immunity Test.	
							Interval.	Result.
19524	Blood..	100 c.c.	1	Subcut.	1 : 500	No reaction....	14 days	Died.
19520*	Blood..	100 c.c.	3	Subcut.	1 : 750	No reaction....	14 days	Died.
19525	Blood..	100 c.c.	1	Intraj.	1 : 1,000	No reaction....	14 days	Died
19522	Blood..	100 c.c.	1	Subcut.	1 : 1,000	No reaction....	14 days	Died.
19519	Blood..	100 c.c.	1	Subcut.	1 : 1,500	No reaction....	14 days	Died.

* The original injection was repeated twice at intervals of 4 days and immunity test carried out 14 days after the last injection.

Discussion.—Although the virus could be inactivated by all concentrations of formaldehyde from 1:500 to 1:1,500 in 10 days at 3° C., its injection in 100 c.c. amounts, either subcutaneously or intravenously, did not afford any protection against a subsequent 5 c.c. intravenous injection of virulent O. virus.

In view of these results, it was decided to retest the immunity conferred by the same material which gave such an encouraging result in the previous experiment No. 4, and in addition to ascertain whether any beneficial result would be obtained from 2 injections of that material at an interval of 8 days.

EXPERIMENT 6. (3862 B.)

Object: to confirm the result obtained with horse No. 19472 in experiment No. 4, namely to ascertain whether the same material after storage at 3° C. for 67 days would continue to confer a solid immunity against O. virus and whether a second injection after an interval of 8 days would have any effect.

Method.—The same material as previously used for horse No. 19472 was injected into 2 horses. As the available supply was limited, the dosage was reduced to 50 c.c.

Horse No,	Dose.	No. of Injections.	Formaldehyde Concentrations.	Time and Temp.	Reaction.	Immunity Test.	
						Interval.	Result.
19521	50 c.c.	2 at 8 day interval	1 : 1,000	3° C. for 67 days..	No reaction	14 days	Died after prolonged reaction.
19523	50 c.c.	1	1 : 1,000	3° C. for 69 days..	Slight fever	14 days	Died after prolonged reaction.

Discussion.—The result obtained in the previous experiment No. 4 could not be confirmed, though apparently some immunity was set up as indicated by the deaths at the test for immunity occurring only on the 8th day after infection. It is of interest to note that whereas the virus was only partially inactivated by a concentration of 1 : 1,000 formaldehyde after 9 days at 3° C., it was almost completely inactivated after 67 days, but no immunity sufficient to protect against an intravenous injection of 5 c.c. of O. virus was produced.

The discrepancy in the results obtained must probably be explained by individual variation in the susceptibility of the different animals to horsesickness. This is a peculiarity which is continually encountered in this disease, and is one of the factors which makes immunization so difficult a problem.

The possibility should also be borne in mind that the prolonged action of the formalin on the virus may have rendered the latter almost completely inactive, and thus reduced its immunizing value, although this possibility is not considered likely in view of the results obtained by other authors in similar work.

To complete the work on inactivation of blood virus, it was decided to observe the effect of varying concentrations of formaldehyde at slightly higher temperatures, due regard being paid to the results obtained in experiment No. 4.

EXPERIMENT 7. (S. 3880.)

Object: to determine the effect of varying concentrations of formaldehyde on the O. virus content of defibrinated blood when stored at 25.5-27° C. and to test the immunity produced by the intravenous injection in 100 c.c. amounts of such modified virus, either once or twice, at an interval of 8 days.

Method.—Defibrinated infective blood was obtained in the usual way from horse No. 19435, which showed a typical horsesickness reaction following subinoculation from horse No. 19472 in experiment No. 4. Formalin was added to obtain concentrations in different samples of this blood of 1 part of formaldehyde to 1,000, 1,500, 2,000, and 3,000 parts respectively. Before injection into susceptible horses, this was allowed to stand for 60 hours in a warm room, the temperature of which fluctuated between 25.5-27° C.

Horse No.	Dose.	No. of Injections.	Formaldehyde Concentrations.	Temp. and Time.	Reaction.	Immunity Test.	
						Interval.	Result.
18942	100 c.c. intraj.	2 at 8 day interval	1 : 3,000	25·5–27° C. for 60 hours and 10½ days	Died 17 days after first injection	—	—
19409	100 c.c. intraj.	1	1 : 2,000	25·5–27° C. for 60 hours	No reaction	20 days	Died.
19413	100 c.c. intraj.	2 at 8 day interval	1 : 1,500	25·5–27° C. for 60 hours and 10½ days	No reaction	22 days	Died.
19379	100 c.c. intraj.	1	1 : 1,000	25.5–27° C. for 60 hours	No reaction	22 days	Died.

Discussion.—A concentration of formaldehyde of 1 part to 1,000, 1,500, or 2,000 parts of defibrinated blood when stored for 60 hours at 25.5-27° C. completely inactivates the virus, and after 22 days produced no apparent immunity when injected intrajugularly in doses of 100 c.c.

On the other hand, it would appear that a concentration of 1 to 2,000 only partially inactivates the virus when kept for the same length of time at the same temperature. Partial inactivation is concluded since the recipient horse No. 18942 did not commence to show any temperature reaction until 12 days after the first injection and died only on the 17th day, the disease showing a somewhat prolonged course, but it is not possible to express an opinion on the period of incubation, since it is not clear whether the incidence of horsesickness was due to the first, the second, or the accumulated effect of both injections. At any rate, it is clear that after an interval of 8 days no effective immunity was produced by one injection to protect against another of the same modified virus. With this in view, it was decided to repeat the experiment, using the same material, but giving 3 injections subcutaneously at intervals of 4 days.

EXPERIMENT 8. (S. 3880 A.)

Object: as Experiment No. 7, except that the immunity produced by 3 successive subcutaneous injections at 4-day intervals is to be determined.

Method.—The same material was used as in Experiment No. 7, the only difference being that at the time of the first injection the formaldehyde had been in contact with the virus for 26 days, and as the supply was somewhat limited the dose was reduced from 100 to 50 c.c.

Horse No.	Dose.	No. of Injections.	Formaldehyde Concentration.	Temp. and Time.	Reaction.	Immunity Test.	
						Interval.	Result.
19503	50 c.c. Subc.	3 at 4 day intervals	1 : 3,000	25·5–26° C. for 26 days	No reaction	7 days after last injection	Died.
19516	50 c.c. Subc.	3 at 4 day intervals	1 : 2,000	25·5–26° C. for 26 days	No reaction	7 days after last injection	Died.
19510	50 c.c. Subc.	3 at 4 day intervals	1 : 1,500	25·5–26° C. for 26 days	No reaction	7 days after last injection	Died.
19511	50 c.c. Subc.	3 at 4 day intervals	1 : 1,000	25·5–26° C. for 26 days	No reaction	7 days after last injection	Died.

Discussion.—Although concentrations of 1 part of formaldehyde to 1,000, 1,500, 2,000, and 3,000 parts of defibrinated blood completely inactivated the O. virus content when stored for 26 days at a temperature ranging between 25.5 and 27° C., no immunity was produced by 3 successive subcutaneous injections at 4-day intervals in 50 c.c. amounts.

At this stage it was decided that there was but a remote possibility of producing an efficient vaccine by the formalization of infective blood, since the antigenic properties of this material appear to be distinctly limited. This conclusion would appear to be justified from the results obtained by other workers engaged in similar attempts to produce efficient vaccines by the same method, in addition to our own results. Consequently, it was agreed that the work should be continued, using, however, emulsified infective tissue, principally spleen, but, as the economic advantages of using only blood are so great that they cannot be discarded without the fullest investigation, some of the subsequent work was duplicated to include both tissue and blood virus.

EXPERIMENT 9. (S. 3986.)

Object: to determine the effect of a concentration of 1 part of formaldehyde to 2,000 parts of an emulsion of infective spleen, and to test the immunity conferred by either 1, 2, or 3 injections of this material.

Method.—Horse No. 19639 was killed when *in extremis* during the reaction to O. virus. A 20 per cent. emulsion of spleen in normal saline was made as described before, and formalin added until a concentration of 1 part of formaldehyde to 2,000 parts of emulsion was obtained. This was incubated at 37° C. for 48 hours and then placed in cold storage at 3° C. for 7 days before injection.

Horse No.	Dose and No. of Injections.	Formaldehyde Concentrations.	Time and Temperature.	Reaction.	Immunity Test.	
					Interval.	Result.
19659	30 c.c. subcut.	1 : 2,000	48 hours at 37° C., then 7 days at 3° C.	No reaction...	7 days.....	Died.
19656	30 c.c. subcut. repeated once after 7 days	1 : 2,000	48 hours at 37° C., then 7 days at 3° C.	Slight reaction	7 days after 2nd injection	Died.
19680	30 c.c. subcut. repeated twice at 7 days interval	1 : 2,000	48 hours at 37° C., then 7 days at 3° C.	No reaction...	7 days after 3rd injection	Died.

Discussion.—Although the virus contained in the spleen was inactivated to such an extent that its injection subcutaneously could be undertaken with complete safety, neither 1, 2, or 3 injections produced an immunity sufficient to protect against a subsequent injection of O. virus. Nor could any partial immunity, as indicated by a prolonged period of incubation, or lengthened course of the disease, be demonstrated.

Since it appears that the complete inactivation of the virus is accompanied by total destruction of all antigenic properties, it was decided to continue the work a step further, i.e. to ascertain whether repeated injections of spleen virus treated with progressively lower concentrations of formaldehyde would be accompanied by more favourable results.

EXPERIMENT 10. (S. 4035.)

Object: to determine the immunological value of repeated injections of emulsified spleen virus decreasingly inactivated by progressively lower concentrations of formaldehyde.

Method.—Emulsified spleen virus was obtained from Horse No. 19765, as before. This was divided into several portions to which formalin was added to produce a final concentration of 1 part of formaldehyde to 2,000, 3,000, 4,000, 8,000, and 10,000 parts of a spleen emulsion respectively. This material was incubated at 37° C. for 48 hours, and then placed in cold storage at 3°. The first injection was not made until 7 days at 3° had elapsed. All injections were made subcutaneously and the dose was 30 c.c.

Results.—The results are clearly indicated in the table on page 94, which indicates the day on which the injection was made, the concentration of formaldehyde in the spleen emulsion injected on that day, the result of each injection, and the final test of immunity.

Discussion.—The results obtained in this experiment indicate first and foremost that, by repeated injections of emulsified spleen virus treated with decreasing concentrations of formaldehyde, it is possible to produce an immunity adequate to protect the animal against the subsequent intravenous injection of 5 c.c. of virulent O. virus.

It would be absurd to suggest that these 6 horses were immune before the commencement of the experiment, since they were drawn haphazard from the same batch of animals as those which died of horsesickness in this and the previous experiment. Also the history of these animals made it practically certain that they had no previous immunity against horsesickness.

In addition to the production of a solid immunity, several points of great importance are brought out as a result of this and previous experiments, namely:—

1. A concentration of 1 part of formaldehyde to 2,000 parts of a 20 per cent. spleen emulsion after incubation at 37° C. for 48 hours and storage at 3° C. for 7 days inactivates the virus, so that in only 2 out of 9 horses did its injection cause a slight fever reaction with no clinical symptoms beyond slight inappetence.

2. Whereas an injection of 30 c.c. of this material subcutaneously does not produce a solid immunity against O. virus even when repeated on two occasions at intervals of 7 days, an apparent partial immunity is produced sufficient to protect against spleen virus treated in a similar manner with lower concentrations of formaldehyde.

3. No test was carried out with material treated with a concentration of 1 in 2,000 or 1 in 4,000, but in concentration of 1:8,000 and 1:10,000 formaldehyde has no demonstrable effect upon the virulence of the virus.

4. Bacterial sterility tests which have not been specifically mentioned in the context indicate that with ordinary reasonable precautions it is possible to produce a sterile product with concentrations of formaldehyde up to 1:4,000, but in lower dilutions the growth of putrefactive organisms is not completely inhibited.

Horse No.	First Injection.	Number of Days after First Injection.								Immunity Test.	
		7 days.	14 days.	21 days.	28 days.	35 days.	42 days.	49 days.	56 days.	Interval since last Injection.	Result.
19670	1 : 2,000.....	1 : 2,000	1 : 2,000	—	—	1 : 3,000	1 : 4,000	—	1 : 8,000	14 days	No re-
	No reaction	No reaction	No reaction			No reaction	No reaction		No reaction		action.
19662	1 : 2,000.....	1 : 2,000	—	—	1 : 3,000	1 : 4,000	—	1 : 8,000	—	14 days	No re-
	Slight fever	No reaction			No reaction	No reaction		Sl. fever			action.
19664	1 : 2,000.....	—	—	1 : 3,000	1 : 4,000	—	—	1 : 10,000	—	14 days	No re-
	No reaction			No reaction	No reaction			No reaction			action.
19643	1 : 2,000.....	1 : 3,000	1 : 4,000	1 : 8,000	—	—	—	—	—	14 days	No re-
	No reaction	Sl. fever	No reaction	No reaction							action.
19650	1 : 2,000.....	1 : 3,000	1 : 4,000	1 : 8,000	—	—	—	—	—	14 days	No re-
	No reaction	No reaction	No reaction	No reaction							action.
19655	1 : 2,000.....	1 : 3,000	1 : 4,000	—	1 : 10,000	—	—	—	—	14 days	No re-
	Slight fever	No reaction	No reaction		No reaction						action.
19788	1 : 8,000.....	—	—	—	—	—	—	—	—	—	—
	Died H.S.										
19764	1 : 10,000.....	—	—	—	—	—	—	—	—	—	—
	Died H.S.										
Control	Pure O. virus.	—	—	—	—	—	—	—	—	—	—
19663	Died H.S.										

With these very promising results in mind it was decided immediately to commence a much more comprehensive experiment, involving 30 horses, to confirm the findings, and, if possible, to throw some light on the mechanism of the formaldehyde action on the virus.

EXPERIMENT 11. (S. 4067.)

Object: to determine the immunological value of repeated injections of inactivated emulsified infective spleen emulsion, inactivated by progressively lower dilutions of formaldehyde.

Method.—Virulent spleen emulsion was obtained from virus horses as before. This was divided into numerous portions to each of which formalin was added to produce a final concentration of 1 part of formaldehyde to 1,000, 2,000, 3,000, 4,000, 6,000, 8,000 and 10,000 parts of tissue emulsion respectively. This material was incubated at 37° C. for 48 hours and then placed in cold storage at 3° C. until required for use. The first injection was only made after 8 days' storage. All injections were made subcutaneously in 30 c.c. doses.

Note.—Dilutions 1:8,000, 1:10,000 were not used owing to obvious putrefaction. It will be seen that Group A (4 horses) served the purpose of acting as a pilot group for the remainder of the horses (26) in the experiment. The object of this was to reduce possible losses to a minimum should any unforeseen mortality occur. It was intended that Group B (16 horses) should receive 7 injections at weekly intervals and Group C (10 horses) 5 injections, but force of circumstances necessitated modification of this plan of procedure from time to time.

Results.—The results are given in the table on pages 96 to 99.

Discussion of Results.—In Group A three out of four horses survived. One horse No. 19749 was destroyed 7 days after the temperature following the horsesickness reaction had returned to normal. In all probability had the phlegmosis of the limbs not developed this animal would have recovered, in which case it would have been completely immune to O. virus. Apparently the reaction was due to the 1:4,000 vaccine and not to the 1:6,000 vaccine.

The other three horses showed reactions, but on immunity test were found to have a solid immunity against O. virus.

In Group B, 5 horses died out of 16. Of these, one (No. 19754) was killed apparently by the 1:3,000 vaccine or as a result of the injection of 1:4,000 vaccine being superimposed upon a horsesickness fever reaction (page 102, fig. 3): two horses (Nos. 19755 and 19771) were killed by the 1:6,000 vaccine. The remaining 11 horses withstood all injections up to the 1:6,000 vaccine and subsequently proved to be immune to O. virus.

In Group C 5 horses died. Of these, two (Nos. 19775 and 19778) were killed apparently by the 1:3,000 vaccine, two (Nos. 19776 and 19779) by the 1:4,000 vaccine, and one (No. 19781) by the 1:6,000 vaccine. The remaining five horses subsequently proved to be immune to O. virus.

Group.	Horse No.	First Injection.	Number of Days after First Injection.								Immunity Test.	
			7 days.	14 days.	21 days.	28 days.	35 days.	42 days.	49 days.	65 days.	Interval after last Injection.	Result.
A	19746	1 : 1,000. . . . No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	1 : 6,000* No reaction	—	—	—	—	39 days	No reaction.
	19747	1 : 1,000. . . . No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	1 : 6,000* No reaction	—	—	—	—	39 days	No reaction.
	19748	1 : 1,000. . . . No reaction	1 : 2,000 Thermal reaction	1 : 3,000 Fever reaction	1 : 4,000 No reaction	1 : 6,000 No reaction	—	—	—	—	39 days	No reaction.
	19749	1 : 1,000. . . . No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	1 : 6,000† Horse-sickness reaction and Dikkop	—	—	—	—	—	—
B	19744	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* Slight fever	—	—	31 days	No reaction.
	19750	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* Horse-sickness slight	—	—	31 days	No reaction.
	19751	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 H.S. from 4th–13th day	1 : 4,000	—	1 : 6,000* Slight thermal	—	—	31 days	No reaction.
	19752	—	1 : 1,000 Fever from da	1 : 2,000 6th to 11th y.	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* Fever	—	—	31 days	No reaction.

B (*contd.*)	19753	—	1 : 1,000 No reaction	1 : 2,000 Sl. fever	1 : 3,000 Sl. fever	1 : 4,000 No reaction	—	1 : 6,000* Sl. fever	—	—	31 days	No reaction,
	19754	—	1 : 1,000 Sl. fever	1 : 2,000 Fever	1 : 3,000 Fever	1 : 4,000 Died, 30 days Horse-sickness	—	—	—	—	—	—
	19755		1 : 1,000 No reaction	1 : 2,000 Sl. fever	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* Died horse sickness day 50	—	—	—	—
	19756	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* No reaction	—	—	31 days	No reaction.
	19757	—	1 : 1,000 No reaction	1 : 2,000 Sl. fever	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000* Fever	—	—	31 days	No reaction
	19758	—	1 : 1,000 Fever from 6th to 14th	1 : 2,000 pani ed by Strang	1 : 3,000 day accom-	1 : 4,000 les. No reaction	—	1 : 6,000* Sl. fever	—	—	31 days	No reaction.
	19759	—	1 : 1000 No reaction	1 : 2,000 Sl. fever	1 : 3,000 No reaction	1 : 4,000 Sl. fever	—	1 : 6,000* Fever	—	—	31 days	No reaction.
	19760	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 Marked fever	—	1 : 6,000* No reaction	—	—	31 days	No reaction.
	19761	—	1 : 1,000	1 : 2,000 Marked feve	1 : 3,000 r reaction fr	1 : 4,000 om 17th- 28th day.	—	1 : 6,000* Died horse sickness day 45	—	—	—	—
	19769	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 Died horse sickness day 40	—	—	—	—	—	—
	19770	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 Sl. fever	1 : 4,000 No reaction	—	1 : 6,000 No reaction	—	—	31 days	No reaction.
	19771	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	—	1 : 6,000 Died horse sickness day 49	—	—	21 days	—

* Developed large abscess at site of inoculation following this injection and this probably accounts for fever reactions.
† Developed extensive phlegmosis of legs by extension from abscessation at site of injection and destruction was imperative.

Group.	Horse No.	First Injection.	No. of Days after First Injection.								Immunity Test.	
			7 days.	14 days.	21 days.	28 days.	35 days.	42 days.	49 days.	65 days.	Interval after last Injection.	Result.
C	19772	—	1 : 1,000 Sl. fever	1 : 2,000 Sl. fever	1 : 3,000 No reaction	1 : 4,000 No reaction	—	—	—	1 : 6,000 Marked fever	21 days	No reaction.
	19773	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 Marked fever	—	—	—	1 : 6,000 Marked fever	21 days	No reaction.
	19774	—	1 : 1,000 No reaction	1 : 2,000 Fever from	1 : 3,000 18–35th day Strangles.	1 : 4,000 complicated	—	—	—	1 : 6,000 No reaction	21 days	No reaction.
	19775	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 Died of hor	1 : 4,000 se sickness on da y 28.	—	—	—	—	—	—
	19776	—	1 : 1.000 Fever from	1 : 2,000 8–17th day.	1 : 3,000 No reaction	1 : 4,000 Died horse sickness 37th day	—	—	—	—	—	—
	19777	—	1 : 1,000 No reaction	1 : 2,000 No reaction	1 : 3,000 Fever from	1 : 4,000 27-42th day	—	—	—	1 : 6,000 No reaction	21 days	No reaction.
	19778	—	1 : 1,000 No reaction	1 : 2,000 Fever from	1 : 3,000 14-24th day	1 : 4,000 Died horse sickness 23rd day	—	—	—	—	—	—
	19779	—	1 : 1,000 Fever from	1 : 2,000 7–19th day	1 : 3,000 No reaction	1 : 4,000 Died horse sickness 43 day	—	—	—	—	—	—
	19730	—	1 : 1,000 Fever from	1 : 2,000 7–21st day	1 : 3,000	1 : 4,000 Horse sickness from 35-39 day	—	—	—	1 : 6,000 No reaction	14 days	No reaction.
	19781	—	1 : 1,000 Fever	1 : 2,000 from 11–22n	1 : 3,000 d day	1 : 4,000 No reaction	—	—	—	1 : 6,000 Died horse sickness 71 day	—	—

6	Controls	19574	—	1 : 2,000 No reaction	1 : 3,000 No reaction	1 : 4,000 No reaction	1 : 6,000 Horsesickness reaction and Dikkop	—	Untreated emulsified spleen; fever reaction	—	—	35 days	No reaction.
		19766	—	—	1 : 3,000 No reaction	1 : 4,000 No reaction	1 : 6,000 No reaction	—	—	—	—	14 days	No reaction.
		19767	—	—	—	1 : 3,000 No reaction	1 : 4,000 Severe horse sickness	—	—	—	—	14 days	Died but death not due to horse sickness.
		19784	—	—	—	—	1 : 4,000 Died horse sickness day 6	—	—	—	—	—	—
		19785	—	—	—	—	Pure O. virus. Died horse sickness day 8	—	—	—	—	—	—
		19786	—	—	—	—	—	—	—	—	1 : 6,000 Died horse sickness day 5	—	—
		19789	—	—	—	—	—	—	—	—	1 : 6,000 Destroyed *in extremis*, day 5.	—	—

* Developed large abscess at site of inoculation following this injection and this probably accounts for fever reactions.
† Developed extensive phlegmosis of legs by extension from abscessation at site of injection and destruction was imperative.

In the Control Group, (*a*) 1 horse (19785) was used as a test for O. virus and was destroyed *in extremis* for further vaccine production.

(*b*) In 1 horse (19574) the first injection of 1:1,000 vaccine was omitted. The animal survived all the remaining injections and developed a solid immunity.

(*c*) In 2 horses (Nos. 19767 and 19766) the first two injections, namely, 1:1,000 and 1:2,000 vaccine were omitted. Of these, 19767 received only 1:3,000 and 1:4,000 vaccine, showed a severe horsesickness fever reaction, and died of broncho-pneumonia and acute gastritis 9 days after being injected with O. virus to test its immunity. Horse No. 19766, however, survived the 1:3,000, 1:4,000, and 1:6,000 injections and subsequently proved immune.

(*d*) In 1 horse (19784) immunization was commenced with 1:4,000 vaccine, which proved fully virulent.

(*e*) In 2 horses (19786 and 19789) the only injection was 1:6,000 vaccine. The reaction produced by the injection of tissue virus inactivated by this concentration of formaldehyde cannot be distinguished from that produced by the injection of untreated O. virus.

The final experiment was not so successful as was anticipated. A mortality of 11 out of 30 is too high for all practical purposes. The definite impression was gained that the chief fault was that the interval between the injections was too short (1 week). The result was that the injection of a partially inactivated vaccine was superimposed upon a horsesickness fever reaction resulting in the death of the animal. (See fig. 3, horse No. 19754.) On the other hand, the interval should not be too long; in that case the temporary immunity produced by one dose may decrease to such an extent that it will not protect against the next (c.f. horse No. 19781). It was thought that the optimum interval between injections would be 14 days.

Conclusions.—(1) It is possible to produce a solid immunity against horsesickness in horses by the subcutaneous injection of virulent spleen emulsion which is decreasingly inactivated by progessively lower concentrations of formaldehyde.

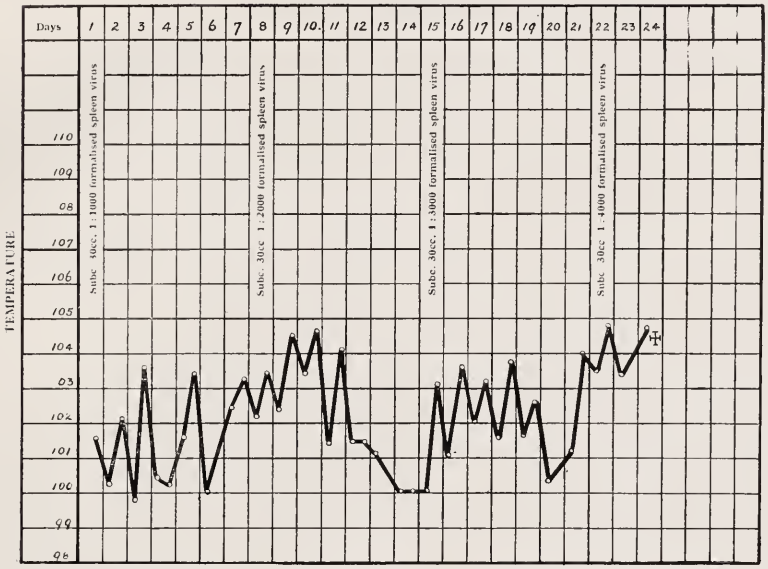
(2) The minimum concentration of formaldehyde to produce an inactivated virus which may be injected into susceptible horses with complete safety and yet produce a partial immunity has not been definitely fixed. A concentration of 1:1,000 seems perfectly safe, some horses survive the 1:2,000 and 1:3,000 vaccine, but 1:4,000 is definitely insufficiently inactivated.

(3) Horses which resist an injection of tissue virus inactivated by a formaldehyde concentration of 1:6,000 prove, without exception, to be completely immune to O. virus.

(4) In spite of the extreme difficulty amounting almost to an impossibility of developing a technique which will ensure complete bacterial sterility during all the operations attendant upon the preparation of the vaccine, a concentration of 1 part of formaldehyde to 4,000 parts of emulsion can be relied upon to inhibit the growth of those organisms which are a probable source of contamination. The sterility of the 1:6,000 product cannot be relied upon, and its possible toxicity should be determined by small animal inoculation.

(5) The immunity produced by the inactivated vaccine is probably transient. (See fig. 4.) To produce a lasting immunity, the final injection should be untreated virulent virus (c.f. horse Nos. 19772 and No. 19773).

Fig. 3. Horse 19754.



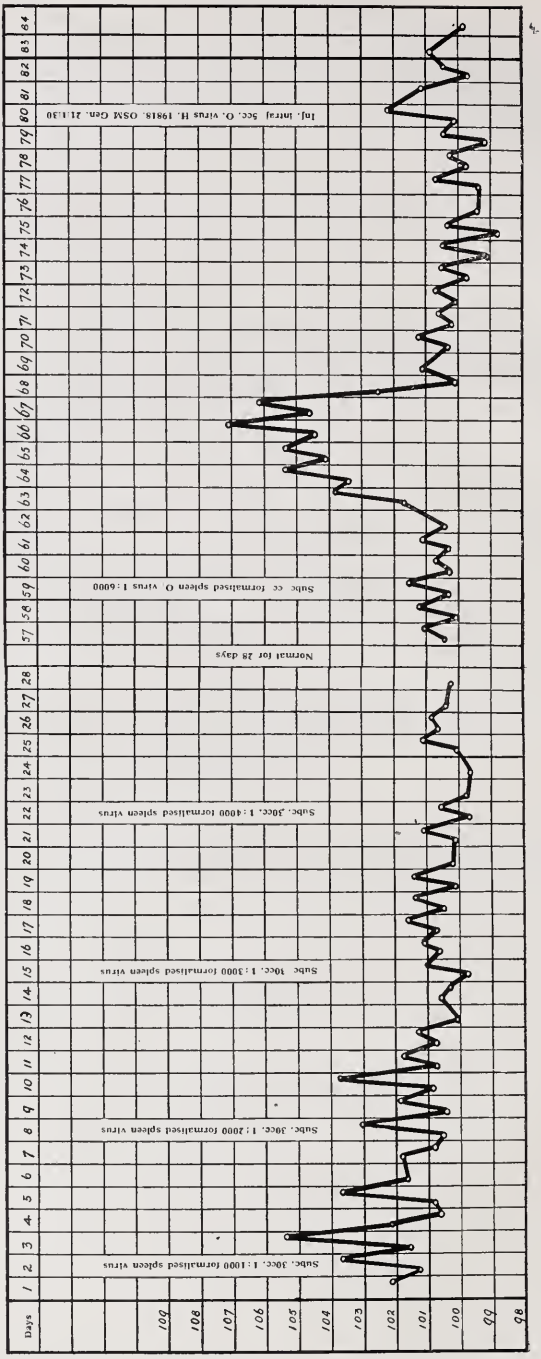

Days
98
99
100
101
102
103
104
105
106
107
108
109
Subc. 30cc. 1 : 1000 formalised spleen virus
Subc. 30cc. 1 : 2000 formalised spleen virus
Subc. 30cc. 1 : 3000 formalised spleen virus
Subc. 30cc. 1 : 4000 formalised spleen virus
Normal for 28 days
Subc. cc formalised spleen O virus 1 : 6000
Inj. intraj. 5cc. O. virus H. 19818. OSM Gen. 21.1.30

FIG. 4.

Section III.

Bacterial Diseases.

E. M. Robinson.. The Bacteria of the *Clostridium botulinum C* and *D* types.

G. Martinaglia.. A Strain of *Mycobacterium tuberculosis* from the Giraffe and Further Observations of *M. tuberculosis* from the Koodoo and the Duiker.

J. G. Bekker ... A Note on the Addition of Glycerine, as a Preservative, for Anthrax Spore Vaccine.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

The Bacteria of the *Clostridium Botulinum C* and *D* types.

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Thesis approved for the D.V.Sc. Degree of the University of South Africa.

INTRODUCTION.

During the last seven years a great deal of information has been obtained about certain bacteria which for the sake of convenience will be called the *Clostridium botulinum C* and *D* types. The question as to whether some of the bacteria to be described should be classified as new types or given the general designation of C or D type will be discussed later in the course of this thesis. Since the investigations of Von Ermengem (1912), in Germany, commenced in 1895, a great deal of research has been carried out by workers in human medicine on the bacteria of the *Clostridium botulinum A* and *B* types. This has been very ably summarized by K. F. Meyer (1928) in his article on " Botulismus " in the latest edition of Kolle and Wassermann's " Handbuch der pathogenen Mikroorganismen." To the work of Meyer and his co-workers is due most of our recently acquired knowledge of botulism.

It was not until 1922, however, that it was realized that other types of the *Cl. botulinum* than the A and B existed. The first organism of the C type to be described was isolated by Bengtson (1922). It was obtained from larvae of *Lucilia caesar* taken from the carcass of a chicken which had died of " limber-neck," the name given by American writers to a disease in poultry in which the chief symptom is that of paralysis. The term " limber-neck " is somewhat loosely used and may embrace a number of conditions in which symptoms due to affection of the nervous system appear, but there is very little doubt that the majority of cases of " limber-neck " are in reality true botulism. The toxin of this organism, a strict anaerobe, when given *per os* or by subcutaneous inoculation to small animals such as rabbits, guinea-pigs or mice, produced symptoms indistinguishable from those seen in botulism due to toxins of the A or B types. It was found that the toxin was not neutralized by the antitoxins against the latter two types.

Bengtson gave a detailed description of the organism isolated by her, including the effect of the toxin on various types of laboratory animals, and, in addition, on pigeons, chickens, and monkeys. The organism has been classified in Bergey's Determinative Bacteriology as *Clostridium luciliae*, the suitability of which name will be discussed later.

Seddon (1922) published a description of an organism isolated by him from a decomposed bone from an area in Australia, where the so-called " Tasmanian Midland Disease " occurred. This disease is known under other names in different parts of Australia, and is essentially a type of botulism due to eating toxic carcass material. Seddon (1925) was subsequently able to demonstrate the toxin of the same organism in material such as decomposed rabbit carcasses, and to show that such toxic material had a wide distribution. He found that the toxin of his organism, which closely resembled the *Clostr. botulinum A* and *B* in morphological and some cultural characteristics, was not neutralized by antitoxins against the A and B types. Not being aware at the time of the work of Bengtson, he described the organism as *Bacillus parabotulinus*, considering it to be a new species. This it may eventually prove to be but comparative tests seem to show that it varies but little from the *Cl. botulinum C* in its main features though, as will be seen later, toxin-antitoxin tests show that antitoxin against Seddon's organism may not neutralize *Cl. botulinum C* toxin.

Graham and Boughton (1923) gave a description of an organism isolated by them from fowls affected with the disease previously mentioned as " limber-neck." They mentioned that the type of " limber-neck " associated with the C type of botulinus organism was not so fatal as that associated with the A and B types as described by Hart (1920) and others in the United States of America. They suggested that for the C type toxin to produce symptoms in poultry some lowering of the resistance was necessary, such as might be caused by parasitic infection or certain bacterial diseases. The organism was isolated from the intestinal contents of affected fowls and in one case it was actually shown that extracts of the intestinal contents would produce symptoms of botulism in guinea-pigs.

From the intestinal contents of a horse and of a steer that had died showing symptoms of botulism, Graham and Boughton were able to isolate an organism corresponding morphologically, culturally and by comparative toxin-antitoxin tests with their *Cl. botulinum C*.

The *Cl. botulinum C* of Bengtson and that of Graham and Boughton correspond in most of their characteristics, and there is little doubt that they are of the same type and cannot be differentiated.

Theiler (1920) obtained impure toxic cultures of an organism of the botulinus type from carcass material which had produced " lamsiekte " in cattle in South Africa. This disease is a type of botulism causing heavy mortality in cattle in certain parts of South Africa, and is associated with the occurrence of osteophagia due to phosphorus deficiency in the vegetation. When eventually isolated

in pure culture (1927) the organism was found to be related to the C type, definitely not the A or B type, of the *Cl. botulinum*, but comparative toxin-antitoxin tests showed that it did not correspond to either the A, B or C types. Provisionally the name *Cl. parabotulinum bovis* was given to it but, as suggested by Meyer and Gunnison (1929) and Weinberg (1927), a better name for it would be *Cl. botulinum D*.

This *Cl. botulinum D* resembles the C type more than the A or B judging by its morphological and cultural characteristics and only on toxin-antitoxin tests can it be differentiated from the C type. A further strain of *Cl. botulinum* has been isolated from carcass material from the same farm as the *Cl. botulinum D* was obtained from, but appears not to correspond exactly to the description of that organism. The comparison will be given under the section of this paper dealing with comparative toxin-antitoxin tests.

Theiler and the writer (1927) described an organism isolated from a rat carcass which had caused botulism in mules at the Veterinary Research Laboratory at Onderstepoort, Pretoria. The cases were very acute and death occurred in a few hours after symptoms were shown. The food was found to have been contaminated by decomposition products from a rat. Saline extracts from the rat's carcass produced symptoms of botulism in guinea-pigs and eventually an organism which produced typical symptoms of botulism as seen in the mules, was isolated from the toxic material. Morphologically and culturally the organism was very similar to the *Cl. botulinum C* and *D*, but could be shown to be definitely not the D type as judged by toxin-antitoxin tests. Recently it has been shown by Graham (private communication) and by the writer, that *Cl. parabotulinum equi*, as the organism from the rat carcass was named, cannot be differentiated from *Cl. botulinum C* when compared by toxin-antitoxin tests. As will be seen from the other tests carried out by the writer, the *Cl. parabotulinum equi* may have to be considered as a variant of the C type.

No other pure culture strains of the *Cl. botulinum* type have been available in this work, but the writer has been fortunate in obtaining a variety of strains in impure culture from various sources. The amount of labour involved has precluded the possibility of the writer with the time at his disposal, attempting to isolate these strains in pure culture. It is, therefore, possible that they may have more than one type of toxin-producing anaerobe in them, but the toxin-antitoxin tests do not suggest that this is the case, though as will be seen in the discussion of their classification by toxin-antitoxin tests, the results in some cases were not completely uniform.

These impure cultures include three strains from carcass material or intestinal contents of cattle from a farm where lamsiekte was constantly occurring. Three other strains were obtained from the carcasses of rats which had been allowed to decompose in closed jars at this Laboratory. A reference is made by the writer (1929) to these latter three strains in an article in the 15th Report of the Director of Veterinary Services. It will be seen from the toxin-antitoxin tests in another part of this article that they are *Cl. botulinum*

D types. Several other strains were encountered. One (No. 628) was obtained from the caecal contents of a horse which died of botulism on a farm near Johannesburg. Two horses actually died, showing very acute symptoms of botulism and it is of interest to note that the owner had been carrying out an intensive campaign for the destruction of rats in his forage store. Another strain (No. 11) was obtained from the carcass of a kitten which had died in some hay which had been associated with an outbreak of botulism affecting several donkeys and a horse in a contractor's stables in Johannesburg. Strain 628 was a C type and 11 apparently a D type. These cases are described in detail in the article by the writer (1929) previously referred to. From some further cases described in the same article three more strains were obtained.

During December, 1928, eighteen water birds, mainly ducks, died in the Pretoria Zoo shortly after a very heavy rainfall. The birds were all in one particular pond, and the symptoms shown were those of " limber-neck." From the caecal contents of several of these birds, toxic cultures were obtained which produced typical symptoms of botulism in guinea-pigs.

Early in 1929, carcass material, chiefly decomposed bones, was obtained from sheep which had died of a condition resembling lamsiekte as seen in cattle. These sheep had developed osteophagia as a result of phosphorus deficiency in the vegetation, and the bones were obviously lighter and more brittle than they normally should have been. From these bones, highly toxic cultures containing a toxin of the botulinus type were obtained. The strain will be referred to as *Bekker* as it was obtained by Mr. J. G. Bekker, Veterinary Research Officer, from Bredasdorp in the Cape Province. The last strain to be mentioned was from an outbreak of a disease in turkeys which caused a heavy mortality and the birds showed symptoms which very closely resembled those of botulism. The writer did not actually see the birds during life and was only able to obtain one dead one for investigation. From the caecum of this bird a highly toxic culture was obtained which produced typical symptoms of botulism in guinea-pigs. Extracts of intestinal contents given to the guinea-pigs *per os* did not produce any symptoms.

As emphasized by K. F. Meyer in his article on botulism in the latest edition of Kolle and Wassermann's " Handbuch der pathogenen Mikroorganismen " (1928), to have positive proof that botulinus organisms found in the intestinal contents of animals are associated with botulism in these animals, one must in addition demonstrate actual toxin in the material.

In the writer's cases, it is only claimed that the presence of spores of a botulinus type were present in the intestinal contents of animals or birds which had died with symptoms of botulism.

It is, however, only fair to state that the evidence of the association of organisms of the botulinus C type so far described, with botulism in different animal species has been circumstantial. One refers in particular to the work of Bengtson, Seddon, and Graham. The chain of evidence is rarely complete and in the case of cattle and horses, the demonstration of toxin is difficult when one takes into consideration the enormous bulk of the ingesta.

* In comparing the bacteria of the *Clostridium botulinum C* and *D* types, it will be convenient to consider them under four headings:—

(1) Comparative morphological and cultural characteristics.
(2) Comparative toxicity for different animal species.
(3) Comparison by cross agglutination tests.
(4) Comparison by toxin-antitoxin tests.

COMPARATIVE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS.

Comparison of the bacteria of the *Cl. botulinum C* and *D* types by their morphological and cultural characteristics is of very little use in their classification. One is struck by the great similarity of the various types comprising these groups.

All these types appear to be motile under anaerobic conditions but not under aerobic. In all of them the morphology is very similar, the length being 6-8μ and width .5 to .8μ, though shorter forms are often present. Chain formation is common, but the filaments are rarely long. Most of the bacteria occur singly or in pairs, end to end. In colonies, the organisms are mainly filamentous, whereas in media where gas production occurs such as Hibler or chopped meat, the filaments are less frequent.

In the writer's experience the *Cl. parabotulinum equi* or *C* as it should be called, has always appeared larger than the other types and a number of forms resembling the orgonts of *Cl. chauvoei* have been seen in cultures of this organism. In the case of the C type obtained from the United States of America, it always appeared somewhat smaller than the other types. It is probable that all the types after being kept in culture media for some years become somewhat smaller in appearance.

In all the types the organisms usually only stain positively by gram in young cultures. After about 48 hours incubation, many gram negative forms are present and they become progressively more frequent until in old cultures most of them are gram negative. Spores are not frequent in chopped meat cultures and are very rare in Hibler's medium. In smears made from colonies in deep glucose or liver agar shake cultures, or from cultures in fluid media with a diffusion shell containing particles of meat, the writer has been unable to demonstrate spores microscopically. The spores are usually terminal, less frequently subterminal. Meyer and Gunnison (1928) state that in Rettger's egg medium spores of *Cl. botulinum D* are frequent and are subterminal.

No blackening has been observed in chopped meat medium or Hibler's. There is no actual change to be observed in the meat or brain. In Hibler's medium there is marked gas production and the fluid becomes turbid, the bacteria later settling down on the surface

* Meyer and Gunnison (1929) have recently made a study of some cultures sent to them by the writer and have compared them in addition from the point of view of the occurrence of non-toxic variants.

of the meat. In chopped meat medium there is not so marked a production of gas and the fluid portion of the medium becomes only slightly turbid.

Fluid cultures of all the types the writer has dealt with have exhaled a characteristic slightly sour smell which is, however, not unpleasant.

In other fluid media such as glucose, glucose liver, liver and serum broth, the medium first becomes turbid but the growth rapidly settles out in the course of a few days' incubation leaving the fluid clear.

Sugar Reactions.

According to Meyer (1928) the *Cl. botulinum A* and *B* types ferment the following sugars, glucose, lactose, maltose, laevulose, dextrin, glycerol and salicin. The C type ferments galactose and inositol but not dextrin. *B. parabotulinus* does not ferment sugars.

The writer has carried out sugar tests using a liver broth medium which had been exhausted by coliform bacilli as a basis, with 1 per cent. of the sugar added, incubation being in an anaerobic jar. *Cl. botulinum D* fermented glucose, laevulose, lactose, maltose, glycerine, dulcite and galactose. *Cl. botulinus C* (*equi*) fermented glucose, laevulose, lactose, maltose and glycerine.

In their article on *Cl. botulinum D*, Meyer and Gunnison (1928) mention that the proteolytic properties are low as they are in the C types but somewhat higher than in the latter.

Isolation.

B. Dubowsky and K. F. Meyer (1922) in an article on the methods employed by them in the isolation of the *Cl. botulinum A* and *B* types in the United States give much valuable information as to technique and many valuable hints as to short cuts in the pure culture isolation of these particular types. For the isolation of the *Cl. botulinum C* and *D* types the deep glucose agar or glucose liver agar shake culture (Vignal) is the most satisfactory method which has so far been devised. The C and D types produce fluffy colonies like pieces of cotton wool teased out. In glucose agar they do not show a dense central portion but in glucose liver agar they do. The latter medium is usually fragmented whereas in glucose agar one may see the colonies simply packed in the tube without any fragmentation.

On the surface of solid media the writer has found great difficulty in getting the various types to colonize out. In a few cases where colonies were obtained they were of the rhizoid type described by Meyer and Gunnison (1928).

In all the types the writer has collected, non-toxic colonies have been encountered, and often they are frequent. Large quantities of these non-toxic cultures have been inoculated into guinea-pigs without producing any symptoms. The writer has tried inoculating

guinea-pigs with several consecutive doses of non-toxic cultures followed after an interval by a dose of homologous toxin in amounts of about 2 to 5 M.L.D's but there was no evidence of any immunity having developed. One may, therefore, consider that these types have completely lost their toxicogenic power. This formation of non-toxic types probably goes on in nature and may account for the falling off in virulence often seen in the botulinus types subcultured continuously.

The writer has made numerous attempts to reactivate these non-toxic types by growing them with a large assortment of bacterial types and in a mixture of bacteria of decomposition, but without result. Guinea-pigs when dosed with non-toxic botulinus cultures and killed a few hours later did not develop any toxin of the botulinus type in their carcasses when allowed to decompose.

In a short article on the isolation of the C types the writer (1927) described the technique employed by him. He found that commencing with actual decomposed carcass material it was fairly easy to reduce the number of bacterial types to about four or five by boiling the cultures from the carcass material for an hour. The spores of the botulinus C and D types resisted such boiling. Subsequently the isolation of the organisms in pure culture was a question of continuous subculturing in shake cultures and testing the colonies for toxicity. The plate method of Sturges and Rettger (1919) using non-sporulating bacteria such as cocci or coliforms was found of some assistance.

The table on page 115 gives a comparison of the various C types and the D one as regards their cultural characteristics, etc. It will be seen that any differentiation on them alone would be very difficult though there are some features in which they differ from the A and B types of the *Cl. botulinum* which are given for the sake of comparison.

COMPARISON BY TOXICITY FOR DIFFERENT ANIMAL SPECIES.

While realizing that toxin-antitoxin and serological tests form the only comparative standard of any real value, it is of interest to note the variation in toxicity of the different varieties of the *Cl. botulinum C* and *D* types. One has to assume that the various authors are referring to recently isolated cultures, as in any type the toxicity is liable to fall off markedly in the course of time, even when constantly subcultured.

In the original article of Bengtson (1922) only the effects of the toxin of *Cl. botulinum C* on certain animal species were mentioned. These included the rabbit, guinea-pig, rat, mouse, monkey, pigeon and chicken. In the article of Graham and Boughton (1923) however, also in relation to the C type, the effects on a bigger variety of animal species were described. As compared with the results of Bengtson, the latter authors found that larger doses were required to produce symptoms in fowls, in one case 71 c.c. of meat wash culture *per os* only apparently killing because the chicken's resistance had been reduced by roup. A feature of the C type of Graham and Boughton as described, was the large amount of culture necessary to produce symptoms in cattle and horses, and from the experimental evidence these animals must be considered as comparatively speaking resistant to that particular toxin.

In his article on the *Bacillus parabotulinus*, Seddon (1922) gives the toxicity of cultures of the organism for a number of animal species but leaves out some of importance such as the pig, goat, dog, cat, rat, and mouse. Theiler and Robinson (1927) give the toxicity of the *Cl. parabotulinum bovis* or *Cl. botulinum D* as it should preferably be called, for the domesticated animal species and small laboratory animals, leaving out the monkey however. This want has been recently supplied by Meyer and Gunnison (1928), so that the list is fairly complete. The marked toxicity for cattle in comparison with horses is noteworthy.

In their description of *Cl. parabotulinum equi*, Theiler and Robinson (1928) give the toxicity for all available species of domesticated and laboratory animals. This organism, as has been mentioned elsewhere (see comparison by toxin-antitoxin tests), is not distinguishable from the *Cl. botulinum C* of Bengtson and Graham and Boughton. The extreme toxicity for horses as compared with other animals may however eventually cause it to be classified as a variety of the C type. It has not yet been tested on monkeys so no comparison can be made with other C types from that point of view.

Recently, as mentioned above, Meyer and Gunnison (1928) have tried both the C and D types as to their toxicity for macacus monkeys by the mouth and express the opinion that their toxicity is much lower than that of either the A or B type of the *Cl. botulinum* and infer from that that their toxicity for man is probably low. So far no outbreaks of botulism in man have been traced to toxins of the C or D types. It is probable that in South Africa such outbreaks would be heard of frequently if these types were very toxic for man, as meat is frequently eaten half cooked or almost raw and from animals which had been dead for some hours before being skinned.

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE BOTULINUS TYPES.

Adapted from Table I in "Der botulismus der Haustiere" Theiler and Robinson (1927).

Type.	Motility.	Morphology.	Staining.	Chopped Meat Medium.	Hibler Medium.	Glucose Agar Shake.	Liver Agar Shake.	Gelatine.	·1 Per cent. Agar.	Serum Broth.	Glucose Broth.	Litmus Milk.	Sugar Media.
Cl. botulinum A..... (Type A 223 Lister Inst.)	Motile under anaerobic conditions	Length, 4 to 9μ Br. ·9 to 1·2μ. Oval spores usually terminal	Gram—easily decolourised	Gas rancid. Small turbidity. Meat blackened	As in chopped meat	Gas. Lens-shaped colonies formed	Gas. Lens-shaped colonies formed	Liquefied	Uniform turbidity	Turbid	Gas, turbidity	Not coagulated. Reduced	Ferment glucose, lactose, levulose, dextrin, glycerol and salicin.
Cl. botulinum B..... (Type B95, Lister Inst.)	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.
Cl. botulinum C..... (From R. Graham, Univ. of Illinois, U.S.A.)	Do.	Length, 5 to 8μ. Breadth ·9μ. Spores oval and terminal. Filaments formed	Gram—forms soon appear	Gas. No turbidity. Meat not blackened	Gas. Turbidity. No blackening	No gas. Fluffy colonies like bits of cotton wool	Gas. Fine fluffy colonies like cotton wool	Do.	Do.	Turbid. Growth settles out soon	No gas. Flocculent growth which settles out	Acid coagulated	As A and B. Dextrin fermented.
B. parabotulinus..... (Seddon) American Museum of Natural History)	Do.	L. 3 to 8μ, Br. 1 μ. Spores oval and sub-terminal	Do. Mainly Gram—in old cultures	Do.	Do.	Do.	Do.	Not liquefied	Do.	Do.	Do.	Do.	No sugars fermented.
Cl. botulinum D..... (Theiler and Robinson) Formerly *Cl. parabotulinum bovis*	Do.	L. 4 to 8μ, Br. ·8 to 1μ Spores oval and terminal short chains frequent	Do.	Do. Sweetish smell	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	No gas. Ferments glucose, levulose, sucrose, lactose, maltose, glycerin, inosite and galactose.
Cl. botulinum C..... (equine type) formerly *Cl. parabotulinum equi*	Do.	L. 4 to 8μ, Br. ·8 to 1μ. Spores terminal. Chains frequent	Do.	Do.	Do.	Do.	Do.	Liquefied	Do.	Do.	Do.	Do.	Gas and acid in glucose, levulose, lactose, maltose, glycerin, dextrin.
Cl. botulinum 334..... (A type probably identical with the C one)		Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	—

COMPARATIVE TOXICITY OF THE BOTULINUS TYPES WITH APPROXIMATE MINIMUM LETHAL DOSES.

Adapted from Table III in "Der Botulismus der Haustiere" (1927) with additions.

Toxicity expressed in approx. M.L.D.'s for the guinea-pig per Kg. body weight subcutaneously.

Type.	Man.	Ape.	Horse.	Ox.	Sheep.	Goat.	Pig.	Dog.	Cat.	Rabbit.	Guineapig.	White Rat.	House Rat.	House Mouse.	Fowl.	Duck.	Pigeon and Ostrich.
Cl. botulinum A (Writer's type was A. 223 Lister Institute)	Very toxic	3 to 30 subc.	6,000 subc. lethal in 4 days	About 15,000 subc.	30 subc.	30 subc.	Not susceptible	Not susceptible	300 to 3,000 subc. (von Ermengem)	1 to 10 c.c. subc. 100 to 1,000 per os	·0003 to ·003 c.c. subc. ·3 to 3 c.c. per os per kg.	Very slightly susceptible	As white rat	·3 c.c. subc.	Large doses per os	Large doses per os	300 to 1,500 subc. in pigeon.
Cl. botulinum B (B. 95 Lister Institute)....	Do.	Do.	1,500 subc. lethal in 12 days	Do.	Do.	Do.	Do.	Do.	Do.	Do.	Do.	—	—	Do.	Do.	—	—
Cl. botulinum C (From R. Graham, University of Illinois, U.S.A.)	—	Small doses subc. large doses per os	40,000 per os	800 Subc. About 60,000 per os not fatal	—	—	Do.	Do.	Not susceptible	Do.	·003 c.c. subc. ·3 c.c. per os per kg.	·3 subc.	—	·3 c.c. subc.	6,000 per os in some cases	—	—
B. parabotulinus (Seddon), American Museum of Natural History	—	—	13 subc.	800 subc. 4,000 per os gave no result	6 subc. 800 per os	About 3 subc.	Do.	Do.	Do.	·001 c.c. subc.	·003 c.c. subc. ·3 to 3 c.c. per os per kg.	—	—	·3 to 1 c.c. subc.	9,000 c.c. or more per os	—	—
Cl. botulinum D (Theiler and Robinson) (*Cl. parabotulinum bovis*)	—	As in Cl. bot. C.	600 subc.	1,500 per os 15 subc.	3 subc.	3 subc.	Do.	Do.	Do.	·001 c.c. per kilo subc. 1 to 3 c.c. per os	·003 c.c. per kilo ·3 to 3 c.c. per os per kg.	300 subc.	—	·3 c.c. subc.	No result	12,000 culture per os	Pigeon 600 subc. Ostrich 6,000 per os.
Cl. botulinum C (equine type). (*Cl. parabotulinum equi*)	—	—	3 subc. 30,000 filt. per os 3,000 unfilt. culture per os	15 subc.	3 subc.	Do.	Do.	Do.	Do.	Do.	Do.	300 subc. No result per os	300 subc. No result per os	—	1,500 subc. No result	Toxic	—
Cl. botulinum 334. (A type probably identical with the C one.)	—	—	—	—	Do.	Do.	—	—	—	1 subc.	Do.	—	—	·3 c.c. subc.	—	—	—

COMPARISON BY MEANS OF AGGLUTINATION TESTS.

The serological classification of the A and B types of the *Cl. botulinum* has been carefully studied by Schoenholz and Meyer (1925), who employed the precipitin, complement fixation and agglutination tests, the latter test being used on a large scale. The results were very satisfactory and proved of great value in identifying types.

Pfenninger (1924) used the agglutination test in the classification of the *Cl. botulinum C* types and the *B. parabotulinus* of Seddon. He compared them with the A and B types, the results bearing out in a striking manner those obtained by the toxin-antitoxin tests.

The writer experienced much difficulty at first in the immunizing of rabbits against the washed cultures of the various botulinus types. It was not found for instance that washing three times in normal saline and subsequently heating to 60° C for one hour was always effective in freeing the bacteria from toxin. With strains of low toxicity, not much difficulty was experienced, but when it came to those with powerful toxins, much difficulty was experienced at first. The writer had many setbacks in his earlier attempts at immunization owing to rabbits dying of botulism after the second or third inoculation with bacteria from highly toxic strains. Later, formolized cultures were used (.4 per cent. Formalin) and even without washing these proved very useful for immunization. The formalin may cause clumps to form, particularly in unwashed cultures, but they do not interfere with the immunizing properties.

In order to obtain the bacteria in bulk for inoculation of animals and for serological tests, the writer used the shell broth method described by McEwen (1926). In this method, which is a modification of the methods using particles of tissue in a fluid medium, the meat is put in a parchment diffusion shell, so that it is not actually mixed with the broth or other fluid medium. The surface of the broth which is usually put up in flasks, is covered with a layer of liquid paraffin. The presence of the meat in the diffusion shell appears to produce the requisite anaerobic conditions, and one gets a heavy growth without the drawback of having meat particles mixed with the bacteria.

The writer has used goats in his later immunization work on account of the smaller risk of losing them, more especially from intercurrent infections, and has found them to give good antibacterial sera. They have the additional advantage of giving larger quantities of serum.

The goats were given five subcutaneous inoculations at weekly intervals of a dense emulsion of bacteria, commencing with 1 c.c. and working up to 5 c.c. The sera remained effective for at least six months when kept in cold storage.

For the tests themselves the writer has not made much use of the ordinary macroscopic agglutination method as generally carried out. The reason for this is that emulsions of the botulinus types, more particularly the C ones show a marked tendency to form small bacterial masses which settle out by themselves fairly quickly,

leaving the medium clear. This tendency was less marked with the A and B types and naturally interfered somewhat with tests done by the ordinary macroscopic method.

It was, therefore, decided to adopt the rapid agglutination method of Huddleson (1926) and referred to by Noble (1927). In the writer's hands this method has given excellent results. The technique is simple, and the actual tests are carried out on a plate of glass divided into small squares instead of using tubes. In the first tests carried out by Huddleson using this method, tubes were used but were discarded in favour of a flat glass surface. Using a sheet of glass, one can see the clumps form more readily and the results can be read much sooner.

The amounts of serum used are the same as for the usual macroscopic test, but a much smaller quantity of a dense emulsion of the bacteria is used. The emulsion should be so dense that using the Gates' nephelometer (1921) the platinum loop disappears from sight at a depth of 4 m.m. The bacteria are suspended in a 12 per cent. solution of sodium chloride. After mixing the serum and emulsion, clumping often takes place at once, even without warning, but one should warm the plate of glass up to blood heat over a flame and then read the result. In less than five minutes the agglutination is usually complete but the test should be read again after fifteen minutes. A series of comparative tests will now be given in tables:

TABLE 1.

CL. BOTULINUM D *antiserum* (Rabbit).

Emulsion.	1/25	1/100	1/200	1/500	1/1000	1/2000	1/5000	1/10000	1/20000
Cl. bot. (333).....	+	±	−	−	−	−	−	−	−
Cl. parabot bovis or *Cl. bot D*.....	++	++	++	++	++	++	±	−	−
Cl. parabot C.....	+	±	−	−	−	−	−	−	−
B. parabot (Seddon)....	+	±	−	−	−	−	−	−	−
Cl. parabot equi (*C*).....	++	+	+	±	−	−	−	−	−

From this table it will be seen that *Cl. botulinum D* antiserum will not agglutinate any except the homologous bacterial emulsion to any marked degree. The organism referred to in the table as *Cl botulinum* 333 was obtained from the carcass of an animal which had died of lamsiekte at Armoedsvlakte, Bechuanaland (see toxin list). From cross toxin-antitoxin tests it appeared to be different to *Cl. parabotulinum bovis D* and the agglutination test shows that they are apparently different organisms.

In the following tables the following symbols have been used:—

++ = very marked agglutination.
+ = definite agglutination.
± = partial agglutination.
− = no agglutination.

TABLE 2.

CL. BOTULINUM D *emulsion.*

Antisera.	1/50	1/100	1/200	1/500	1/2000	1/4000	1/8000	1/16000	1/32000
Cl. botulinum D or *Cl. parabot bovis*.....	++	++	++	++	++	++	+	±	−
B. parabotulinus (Seddon).....	++	++	+	±	−	−	−	−	−
Cl. botulinum C.....	++	++	±	−	−	−	−	−	−
Cl. botulinum B.....	++	+	±	−	−	−	−	−	−

In this table an emulsion of the *Cl. parabotulinum bovis* (*Cl. botulinum D*) was tested against four different antisera. The homologous antiserum was the only one to clump the bacteria to any marked extent.

TABLE 3.

CL. BOTULINUM C *emulsion.*

Antisera.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Cl. botulinum C.....	++	++	++	++	++	++	+	±	±
B. parabotulinus (Seddon).....	++	++	+	±	−	−	−	−	−
Cl. parabot. bovis D....	++	++	++	+	±	−	−	−	−
Cl. botulinum B.....	++	++	+	±	−	−	−	−	−

The antisera used for these tests were the same as those used in table 2 and were made by immunizing goats. Again the homologous serum was the only one to agglutinate to a marked degree. Evidence of some degree of group agglutination is seen in these tests.

TABLE 4.

CL. BOTULINUM B *emulsion.*

Antisera.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Cl. botulinum B.....	++	++	++	++	++	++	+	±	−
Cl. botulinum C.....	++	+	±	−	−	−	−	−	−
Cl. botulinum D (bovis)..	+	+	±	−	−	−	−	−	−
B. parabotulinus (Seddon).....	+	±	−	−	−	−	−	−	−

In this table the evidence of group agglutination is less marked than in the two previous tables. Some variation is to be expected

when using different emulsions and emulsions of the same organism prepared at different times may give slightly varying results.

TABLE 5.

CL. BOTULINUM B *emulsion.*

Antisera (Goat).	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. parabot. bovis or *Cl. botulinum D*	++	+	±	−	−	−	−	−
B. parabotulinus (Seddon)	++	+	±	−	−	−	−	−
Cl. botulinum C	+	±	−	−	−	−	−	−
Cl. parabot. equi (C)	±	−	−	−	−	−	−	−
Cl. bot. A	+	+	±	−	−	−	−	−
Cl. bot. B	++	++	++	++	++	+	±	−

The emulsion in this case was not apparently as agglutinable as that in table 4 but the results correspond very closely.

TABLE 6.

B. PARABOTULINUS *(Seddon) emulsion.*

Antisera (Rabbit).	1/50	1/200	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/24000
Cl. bot. D (bovis)	++	++	++	++	+	±	−	−	−	−
Cl. parabot. equi (C)	++	++	++	++	+	±	−	−	−	−
Cl. botulinum C(1)	++	++	+	±	−	−	−	−	−	−
Cl. botulinum C(2)	++	++	++	++	+	±	−	−	−	−
B. parabotulinus (Seddon)	++	++	++	++	++	++	++	+	±	−

Evidence of group agglutination is well marked in this table. The *Cl. botulinum C* sera were from two different rabbits and one gave a much stronger agglutination than the other.

TABLE 7.

B. PARABOTULINUS *(Seddon) emulsion.*

Antiserum (Goat).	1/50	1/100	1/200	1/500	1/1000	1/2000	1/4000	1/8000	1/16000	1/32000
B. parabotulinus (Seddon)	++	++	++	++	++	++	++	+	±	−
Cl. botulinum C.	++	++	+	±	−	−	−	−	−	−
Cl. botulinum D (bovis)	++	+	+	±	−	−	−	−	−	−
Cl. botulinum equi C	++	++	++	++	+	±	−	−	−	−

The results are more or less the same as in table 6, except that the equi antiserum gave a stronger reaction than the C.

In table 8 a *Cl. botulinum C* emulsion was used, this time including an equi antiserum.

TABLE 8.

CL. BOTULINUM C *emulsion.*

Antiserum (Goat).	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. botulinum C	++	++	++	++	++	+	±	−
B. parabotulinus (Seddon)	+	+	±	−	−	−	−	−
Cl. botulinus D (bovis)	++	++	+	±	−	−	−	−
Cl. botulinus C (equi)	++	++	+	±	−	−	−	−

The equi anti-serum did not agglutinate the emulsion as strongly as the C.

TABLE 9.

CL. BOTULINUM C (EQUI) *emulsion.*

Antiserum (Goat).	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. botulinum D (bovis)	++	++	++	±	−	−	−	−
B. parabotulinus (Seddon)	++	++	++	+	±	−	−	−
Cl. botulinum C (equi)	++	++	++	++	++	++	+	±
Cl. botulinum C	++	++	++	++	+	±	−	−

The results in this table show the close relationship of the C and C (equi) types.

TABLE 10.

CL. BOTULINUM 334 (*C type*).

Antiserum (Goat).	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. botulinum D (bovis)	++	++	+	±	−	−	−	−
Cl. botulinum C (equi)	++	++	++	++	++	+	±	−
Cl. botulinum C	++	++	++	++	++	+	±	−
B. parabotulinus (Seddon)	++	++	++	+	±	−	−	−

This table shows the close relationship of the C, C (equi) and 334 types.

As shown by toxin-antitoxin tests, *Cl. botulinum* 334 is definitely a C type of the *Cl. botulinum*, and the tests in Table 10 therefore confirm the relationship.

TABLE 11.

CL. BOTULINUM B *antiserum (goat).*

Emulsion.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. botulinum B.....	++	++	++	++	++	++	+	±
Cl. botulinum A.....	++	+	±	−	−	−	−	−
Cl. botulinum C.....	++	++	+	±	−	−	−	−
Cl. botulinum C (equi).....	++	+	±	−	−	−	−	−
Cl. botulinum D (bovis).....	++	+	±	−	−	−	−	−
B. parabotulinus (Seddon).....	++	+	±	−	−	−	−	−

With *Cl. botulinum A* antiserum (goat) only the A emulsion was strongly agglutinated.

TABLE 12.

CL. BOTULINUM C *antiserum (goat).*

Emulsion.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Cl. botulinum A.....	+	±	−	−	−	−	−	−	−
Cl. botulinum B.....	++	+	±	−	−	−	−	−	−
Cl. botulinum C.....	++	++	++	++	++	++	++	+	±
Cl. botulinum C (equi)..	++	++	++	++	++	++	+	±	−
Cl. botulinum D (bovis)..	++	+	±	−	−	−	−	−	−
B. parabotulinus (Seddon).....	++	+	±	−	−	−	−	−	−

TABLE 13.

CL. BOTULINUM C (EQUI) *antiserum (goat).*

Emulsion.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Cl. botulinum A.....	++	+	±	−	−	−	−	−	−
Cl. botulinum B.....	++	++	+	±	−	−	−	−	−
Cl. botulinum C.....	++	++	++	++	++	+	±	−	−
Cl. botulinum C (equi)..	++	++	++	++	++	++	+	±	−
Cl. botulinum D (bovis)..	++	++	+	±	−	−	−	−	−
B. parabotulinus (Seddon).....	++	++	++	++	+	±	−	−	−

Table 14.

CL. BOTULINUM D (BOVIS) *antiserum (goat)*.

Emulsion.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800
Cl. botulinum A	+	±	−	−	−	−	−	−	−
Cl. botulinum B	++	+	±	−	−	−	−	−	−
Cl. botulinum C	++	+	±	−	−	−	−	−	−
Cl. botulinum C (equi)	++	++	++	+	±	−	−	−	−
Cl. botulinum D (bovis)	++	++	++	++	++	++	++	+	+
B. parabotulinus (Seddon)	++	++	++	+	±	−	−	−	−

Table 15.

B. PARABOTULINUS *(Seddon) antiserum (goat)*.

Emulsion.	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Cl. botulinum A	++	+	±	−	−	−	−	−
Cl. botulinum B	+	±	−	−	−	−	−	−
Cl. botulinum C	++	++	+	±	−	−	−	−
Cl. botulinum C (equi)	++	++	++	+	−	−	−	−
Cl. botulinum D (bovis)	++	++	+	±	−	−	−	−
B. parabotulinus (Seddon)	++	++	++	++	++	++	++	+

Conclusions.

(1) From the comparative agglutination tests carried out, it will be seen that there is a definite group relationship between certain of the various types dealt with. The close relationshop of the *Cl. botulinum C* (American strain) and the *Cl. botulinum C* (*equi*) is shown as well as that of the *Cl. botulinum* (334) to the other two as demonstrated by toxin-antitoxin tests.

(2) The identity of the *B. parabotulinus* of Seddon with any of the other types was not demonstrated and it would appear that it is a variant of the C type.

(3) The value of the shell broth medium for obtaining large amounts of bacterial emulsions of the C and D types in particular is emphasized.

(4) The rapid agglutination test of Huddleston (1926) can be used for the bacteria of *Cl. botulinum* type and has definite advantages over the longer methods.

COMPARISON BY TOXIN-ANTITOXIN TESTS.

Comparison and classification of the botulinus types by means of toxin-antitoxin tests is probably the best means now available for this purpose. In the case of non-toxic types, or those with very weak toxins, other means of classification must be attempted. In the earlier work on the botulinus bacteria it was not realized that more than one type existed. Even now it is not known whether the original type of von Ermengem (1912) for instance, was an A or a B one, and Bengtson (1922) suggests that it was probably more closely related to the C type isolated by her than to either of the other two. Since the work of Burke (1917), the existence and wide distribution of the B type has been recognized. The C type of Bengtson (1922) was established by the failure of its toxin to be neutralized by A and B type antitoxins, though there were, in addition, certain cultural characters in which the organisms differed, such as the colony form and some biochemical features. Seddon (1922) established the existence of a C type of the *Cl. botulinum* in Australia, in association with a paralysis of cattle (Midland disease of Tasmania) and later (1925) demonstrated the toxin of the organism in mixed cultures from carrion in other parts of Australia. Seddon gave his organism the name of *Bacillus parabotulinus*, a name which has not so far been altered by other workers, but it is obvious that the name cannot be allowed to stand indefinitely in view of the fact that the related types are all classified as *Cl. botulinum* of some type. There does not seem to be any real justification for changing the name *Cl. botulinum C* to *Cl. luciliae* as has been done in Bergey's Determinative Bacteriology.

Weinberg (1927) in a criticism of the nomenclature adopted by Theiler and Robinson (1927) in relation to *Cl. parabotulinum bovis*, suggested that the name *Cl. botulinum D* should be substituted for the one given. Meyer and Gunnison (1929) in a brief descriptive article on the organism called *Cl. parabotulinum bovis* by Theiler and Robinson, suggest that it be called *Cl. botulinum* type D. (Theiler and Robinson). The reason given is that the toxin is not neutralized by the antitoxins of either the A, B or C types and therefore the organism should constitute a fourth or D type. The writer is inclined to agree with this classification, and the organism will therefore be referred to as *Cl. botulinum* type D.

The organism described by Theiler and the writer (1928) in association with certain cases of botulism in horses in South Africa, and given the name *Cl. parabotulinum equi*, may eventually prove not to be a new type. In a personal communication to the Director of Veterinary Services, Dr. R. Graham of the Illinois Agricultural Experiment Station, U.S.A., stated that his type C antitoxin neutralized the toxin of the *Cl. parabotulinum equi*. This observation has recently been confirmed by the writer who has, in addition, been able to show that the C toxin was neutralized by *Cl. parabotulinum equi*. It is difficult, however, to explain the differene in virulence of the South African and American types for animals of the same species as will be noted in the section of this paper dealing with comparative virulence.

The writer's ideas and suggestions in relation to the classification and nomenclature of the botulinus types will be further discussed in the final consideration of our knowledge in relation to them.

In the comparative work on toxins and antitoxins of this group, the following types have been available:—

(1) *Cl. botulinum A* (A 223 Lister Institute).
(2) *Cl. botulinum B* (B 95 Lister Institute).
(3) *Cl. botulinum C* (Graham, U.S.A.).
(4) *B. parabotulinus* (Seddon, Australia).
(5) *Cl. botulinum D. (Cl. parabotulinum bovis).*
(6) *Cl. parabotulinum equi* (Theiler and Robinson, 1927).
(7) *Cl. botulinum* 333 (not yet typed; isolated from Lamsiekte carrion).

The abovementioned toxins were all from pure cultures. The following toxins were available as well, but were from mixed cultures from toxic material. They produced typical symptoms of botulism in guinea-pigs, but the cultures from which they were obtained might have contained more than one type of botulinus organism. From the results in the toxin-antitoxin tests, however, this does not seem to have been the case.

Toxins (mixed bacterial cultures).

(8) Rat 3, (9) Rat 5, (10) Rat 6 — From carcasses of rats allowed to decompose in closed fruit jars.

(11) 334, (12) 335, (13) 335a — From carcasses of cattle dead of lamsiekte at Armoedsvlakte, Bechuanaland.

(14) 628. From an outbreak of botulism in horses on a farm between Johannesburg and Pretoria.

(15) 11. From an outbreak of botulism in donkeys in Johannesburg. The culture was from the carcass of a dead cat.

(16) Zoo strain. (From intestinal contents of wild ducks in Pretoria Zoo.)

(17) Bekker strain. (From carcass material from cases of botulism in sheep at Bredasdorp, Cape Province.

(18) Carlisle strain. (From intestinal contents of turkeys dead of botulism on a farm near Pretoria.

All the toxins both from pure and impure cultures were filtered through a small Seitz filter before use in tests.

Antitoxins.

(1) Combined *Cl. botulinum A* and *B* (Parke Davis & Co.).
(2) *Cl. botulinum B* (from an immunized goat).
(3) *Cl. botulinum C* (from Graham, Univ. of Illinois, U.S.A.).
(4) *Cl. botulinum D*, or *parabotulinum bovis* (lamsiekte carrion).
(5) *Cl. botulinum equi* [botulism in horses, Theiler, Robinson, (1927.)]
(6) Rat 3 strain (from the carcass of a decomposed rat).
(7) 335 strain (from lamsiekte carcass material).

Numbers 6 and 7 are the strains referred to in the toxin list as 8 and 12 respectively.

With the exception of antitoxins (1) and (3) the sera were obtained by hyperimmunizing goats. Rabbits were used at first, provided such small quantities of serum and were so liable to die of intercurrent diseases during the immunization process, that they were later given up in favour of goats.

In an article by the writer (1929) now in the press, the process used in the immunization of goats is described, and may be briefly given here, as the article may not appear in print until after the present article has been presented.

In the case of each toxin where a hyperimmune serum was produced against it, an anatoxin was first made from it by the addition of .4 per cent. formalin and incubation at 37° C. until the toxin lost its toxicity. The period of incubation varied with the virulence of the toxins, the more virulent the toxin the longer the incubation period necessary.

In immunization of a goat, the writer commenced first with anatoxin, using as an initial dose 5 c.c. This was followed after 10 days by a dose of 10 c.c. Ten days later a dose of 20 c.c. was given. A week after this 10 M.L.D.'s of pure toxin were given and subsequently the dose was doubled every week until 2,000 M.L.D.'s were given. The serum of the goat would then usually neutralize up to 500 M.L.D.'s for the guinea-pig in a dose of 1 c.c. Further hyperimmunization was not attempted as the serum was then sufficiently potent for cross toxin-antitoxin tests. During the hyperimmunization process, none of the goats showed any symptoms of botulism. Five different hyperimmune sera were produced and all were very satisfactory.

Graham and Thorp (1929) carried out a number of experiments on the detoxification of toxins of *Cl. botulinum* A, B and C types. They showed that the time necessary for detoxification by formalin was very variable and expressed the opinion that immunization by means of anatoxins might prove a very valuable method.

As regards the interrelation of the A, B and C types of the *Clostridium botulinum*, the first attempt at a classification of them by toxin-antitoxin tests was made by Pfenninger (1924). He was able to establish the identity of various C types and to show that Seddon's *B. parabotulinus* toxin was neutralized by a C type antitoxin. An interesting observation made by Pfenninger was that the antitoxin of the *B. parabotulinus* type neutralized the homologous toxin only and not other C types. As will be seen in the tests to be described, *Cl. parabotulinum equi* toxin is neutralized by C type antitoxin and conversely the C type toxin is neutralized by *Cl. parabotulinum equi* antitoxin. Although, therefore, as judged by toxin-antitoxin tests *B. parabotulinus* and *Cl. botulinum C* are not apparently identical; *Cl. parabotulinum equi* and the C type are.

In the course of a large series of toxin-antitoxin tests carried out by the writer, the observations of other workers have been supported and extended. In all the tests the approximate M.L.D.'s of the toxins were determined just previously.

Table 1. 26/3/26.

Toxin.	Antitoxin.	Results.
Cl. botulinum D, 10 M.L.D.'s..... (*Cl. parabot. bovis*)	*Cl. botulinum D*, 5 c.c.....	Survived.
,, ,, ...	,, ,, 1 ,, ...	,,
,, ,, ...	None.....	Died in 48 hours.
Cl. parabot. equi 2 c.c.....	*Cl. botulinum D*, 5 c.c.....	Died in 72 hours.
,, ,, 2 ,, ...	None.....	,, ,,
B. parabot. (Seddon) 2 ,, ...	*Cl. botulinum D*, 5 c.c.....	,, ,,
,, ,, 2 ,, ...	None.....	Died in 48 hours.
Cl. botulinum C, 2 ,, ...	*Cl. botulinum D*, 5 c.c.....	Died in 96 hours.
,, ,, 2 ,, ...	None.....	,, ,,
Cl. botulinum A 10 ,, ...	*Cl. botulinum D*, 5 c.c.....	Died in 72 hours.
,, ,, 2 ,, ...	,, ,, ...	,, ,,
,, ,, 2 ,, ...	None.....	Died in 96 hours.

From this table the difference of the toxin of the lamsiekte organism (*Cl. botulinum D*) from the other toxins used is clearly established. At the time, other antitoxins were not available but were obtained later, when further tests were carried out. A polyvalent antitoxin against the A and B types of the *Cl. botulinum* was obtained from the Burroughs, Welcome Co., and with it a series of tests was carried out. These results are given in Table 2.

Table 2. 1/9/26.

Toxin.	Antitoxin.	Result.
Cl. botulinum A, 2 M.L.D.'s....	1 c.c. *Cl. botulinum A* and *B*.....	Survived.
,, ,, ...	None.....	Died.
Cl. parabot. equi, 2 M.L.D.'s....	1 c.c. *Cl. botulinum A* and *B*.....	,,
,, ,, ...	None.....	,,
B. parabot. (Seddon), 2 M.L.D.'s	1 c.c. *Cl. botulinum A* and *B*.....	,,
,, ,,	None.....	,,
Cl. botulinum C, 2 M.L.D.'s....	1 c.c. *Cl. botulinum A* and *B*.....	,,
,, ,, ...	None.....	,,
Cl. botulinum D, 2 M.L.D.'s.... (*Cl. parabot. bovis*)	1 c.c. *Cl. botulinum A* and *B*.....	,,
,, ,, ...	None.....	,,

From this table it is clear that, as shown by other workers, the C and Seddon types differ from the A and B, and, in addition, *Cl. botulinum D* and *Cl. parabotulinum equi* (*C*) differ from these types.

Until recently the writer had been unable to make an equi (C) serum in any animal species, but after commencing the anatoxin method of immunization, a good serum was obtained. Most of the work done by the writer on toxin-antitoxin tests has, therefore, been carried out in the last year. The original observations on *Cl. botulinum D* were repeated and confirmed, and a series of comparative tests was carried out with other types.

Recently an efficient antitoxin against the toxin of *Cl. parabotulinum equi* (*C*) has been prepared, and it is of interest to note its effect on *Cl. botulinum C* toxin.

TABLE 3.

Toxin.	Antitoxin.	Result.
Cl. parabot. equi (*C*), 5 M.L.D.'s....	2 c.c. *Cl. parabot. equi C*.....	Survived.
Cl. botulinum C ,, ...	,, ,, ...	,,
Cl. parabot. equi (*C*) ,, ...	None.....	Died.

The two toxins are, therefore, identical. Graham, in a personal communication to the writer, stated that he had found his C type antitoxin would neutralize the *Cl. parabotulinum equi* toxin. The results in Table 3 therefore confirm his observation.

Taking the *Cl. botulinum A*, *B*, *C* and *D* as definite types, an attempt was made to classify the toxins mentioned earlier as having been available for testing. Unfortunately in some cases it was not possible to place them absolutely definitely, some showing slight variations from the stock types.

When only the *Cl. botulinum D* antitoxin was available an attempt was made to classify four strains of the *Cl. botulinum* from carcass material at Armoedsvlakte, Bechuanaland. These are given in the toxin list as follows:—

Cl. botulinum 333 (pure culture).
,, 334 (from impure culture).
,, 335 ,, ,,
,, 355 (A) ,, ,,

The results of the test with these four toxins is given in Table 4.

TABLE 4. (24/2/27.)

Toxin.	Antitoxin.	Result.
Cl. botulinum 333, 2 M.L.D.'s.....	2 c.c. *Cl. botulinum D*.....	Died.
,, 333, 2 ,, ...	None.....	,,
,, 334, 2 ,, ...	2 c.c. *Cl. botulinum D*.....	Survived.
,, 334, 2 ,, ...	None.....	Died.
,, 335, 2 ,, ...	2 c.c. *Cl. botulinum D*.....	Survived.
,, 335, 2 ,, ...	None.....	Died.
,, (*A*) 335, 2 ,, ...	2 c.c. *Cl. botulinum D*.....	,,
,, 335, 2 ,, ...	None.....	,,
,, (*D*) 335, 5 ,, ...	2 c.c. *Cl. botulinum D*.....	Survivied.
(*Cl. parabot. bovis*) ,, 5 ,, ...	None.....	Died.

This result was quite unexpected as the toxins had previously all been thought to be of one type. Two of them, *Cl. botulinum* 334 and 335, may be considered to be identical with *Cl. botulinum D* and the other two to belong to another type.

These tests with the four strains in Table 4 were repeated later with identical results. It was, therefore, decided to attempt the classification of the two strains which were apparently not of the *boris* or D type.

Table 5.

Toxin.	Antitoxin.	Result.
Cl. botulinum 333, 2 M.L.D.'s...	2 c.c. *Cl. parabot equi* (*C*).....	Survived.
„ 334, 2 „ ...	„ „	Died.
„ 335, 2 „ ...	„ „	„
„ (*A*) 335, „ ...	„ „	Survived.
Cl. parabot. equi (*C*), 2 M.L.D.'s....	„ „	„
„ „ 2 „	No antitoxin.....	Died.

The results in this table are the exact opposite of those in Table 4. The toxins 333 and 335 (A) therefore appear to be of the C type.

Using an antitoxin made against *Cl. botulinum* (335 A) one gets the results in Table 6.

Table 6.

Toxin.	Antitoxin.	Result.
Cl. botulinum 334, 2 M.L.D.'s.....	*Cl. botulinum* 335 (*A*), 3 c.c.....	Died.
„ 333, 2 „	„ „	Survived.

This result affords further evidence of the relationship of strains 334 and 335 (A), and that 333 and 334 differ. Using an antitoxin made against the toxin of Rat 6, which will later be shown to be a D type, apparently identical with that of *Cl. parabotulinum boris* (*Cl. botulinum D*), it can be shown that it neutralizes the toxin of *Cl. botulinum* 334 but not that of 333.

Table 7.

Toxin.	Antitoxin.	Result.
Cl. botulinum 334, 2 M.L.D.'s.....	*Cl. botulinum* (Rat 6), 3 c.c.....	Survived.
„ 333, 2 „	„ „	Died.

According to the results obtained from the various tests described, it would appear definite that one is dealing with at least two types of the *Cl. botulinum* on the farm Armoedsvlakte, Bechuanaland, Cape

Province. The farm in question was originally purchased by the Government as being the most suitable place for undertaking research work on "lamsiekte" in cattle, as the mortality from the disease was very heavy before preventive measures against it were introduced.

Five strains of the *Cl. botulinum* from the above farm have, therefore, been classified by toxin-antitoxin tests. One was the original *Cl. parabotulinum boris* strain (D), and the other four were the strains *Cl. botulinum* 333, 334, 335 and 335 (A). It may be of interest to mention that strain 333 was from a guinea-pig whose carcass became toxic when exposed in a wire-cage at Armoedsvlakte, the wire having such a fine mesh that only dust could pass it.

These strains must therefore be classified as follows:—

(1) *Cl. botulinum D* or *Cl. parabotulinum boris.*
(2) *Cl. botulinum* 334 is a *Cl. botulinum D* type.
(3) *Cl. botulinum* 335 is a *Cl. botulinum D* type.
(4) *Cl. botulinum* 333 is a *Cl. botulinum C* type.
(5) *Cl. botulinum* 335 (A) is a *Cl. botulinum C* type.

The three toxins from carcasses of rates which had been allowed to decompose in fruit jars were then tested. Against one of these, the toxin from Rat 6, a powerful antiserum had been made by hyper-immunizing goats. As a preliminary step, therefore, it was thought advisable to try whether all three of the toxins were identical or not by the use of antitoxin for Rat 6 toxin.

TABLE 8.

Toxin.	Antitoxin.	Result.
Cl. botulinum (rat 6), 5 M.L.D.'s....	*Cl. botulinum* (rat 6), 3 c.c.....	Survived.
,, (rat 3) 5 ,, ...	,, ,, ...	,,
,, (rat 3) 1 ,, ...	,, ,, ...	,,
,, (rat 5) 5 ,, ...	,, ,, ...	Died.
,, (rat 5) 1 ,, ...	,, ,, ...	Survived.
,, (rat 6) 5 ,, ...	No antitoxin.....	Died.

The toxins of Rats 6 and 3 were, therefore, apparently identical, but that of Rat 5 was different from the other two. The possibility of the toxin of Rat 5 being a mixture of more than one toxin cannot be overlooked, and, as will be seen from some of the other toxin-antitoxin tests the result with impure toxins and antitoxins made from them have been in some cases rather irregular.

In the next table the results of tests using antitoxin from Rat 6 toxin and various toxins are given. It will be seen that a guinea-pig which received toxin of Rat 3 was not protected, but it probably did not die of botulism. In Table 8 it has definitely been shown that the toxins of Rats 3 and 6 are the same, and the tests have been repeated and confirmed this result.

TABLE 9.

Toxin.	Antitoxin.	Result.
B. parabot. (Seddon), 5 M.L.D.'s....	3 c.c. *Cl. botulinum* (rat 6).....	Died.
Cl. parabot. equi (*C*) 5 „	„ „	„
Cl. botulinum (*A*). 5 „	„ „	„
„ (*B*). 5 „	„ „	„
„ (*D*)		
or		
Cl. parabot bovis 2 „	„ „	Survived.
Cl. botulinum (rat 6) 5 „	„ „	„
„ (rat 3) 5 „	„ „	Died.
„ (rat 5) 5 „	„ „	„
„ (rat 6) 5 „	No antitoxin.....	„

From this table it would appear that the toxin of Rat 6 is of the *bovis* or D type.

Table 10 is practically a repetition of Table 8.

TABLE 10.

Toxin.	Antitoxin.	Result.
Cl. botulinum (rat 6) 10 M.L.D.'s...	3 c.c. *Cl. botulinum* (rat 6).....	Survived.
„ (rat 6) 1 „ ...	„ „	„
„ (rat 5) 10 „ ...	„ „	Died.
„ (rat 3) 10 „ ...	„ „	Survived.
„ (rat 6) 10 „ ...	No antitoxin.....	Died.
„ (rat 6) 1 „ ...	„	„

The results in this table confirm those in Table 8. Two further tables, numbers 11 and 12, are of interest in the typing of the strain from Rat 5.

TABLE 11.

Toxin.	Antitoxin.	Result.
Cl. botulinum (rat 3) 2 M.L.D.'s....	*Cl. botulinum* (335 A).....	Died.
„ (rat 5) 2 „	„ „	Survived.
„ (rat 6) 2 „	„ „	Died.
„ (335 A) 2 „	No antitoxin.....	„

Cl. botulinum (335 A) is of the C type of toxin, therefore one must place the toxin of Rat 5 under this type.

TABLE 12.

Toxin.	Antitoxin.	Result.
Cl. botulinum (rat 3) 2 M.L.D.'s....	*Cl. botulinum* (rat 6), 3 c.c.....	Survived.
,, (rat 5) 2 ,, ...	,, ,, ...	Died.
,, (rat 6) 2 ,, ...	,, ,, ...	Survived.
,, (rat 6) 2 ,, ...	No antitoxin.....	Died.

The results in Table 12 confirm those in Table 10, and show that even in the amount of 2 M.L.D.'s the toxin of Rat 5 is not neutralized by *Cl. botulinum* (Rat 6) antitoxin. Rat 6 toxin appears definitely to be a D type. The toxins of the three rats may, therefore, be classified as follows:—

Cl. botulinus (Rat 6) is a *Cl. parabotulinum bovis* or *Cl. botulinum D* type.

Cl. botulinum (Rat 3) is a *Cl. parabotulinum bovis* or *Cl. botulinum D* type.

Cl. botulinum (Rat 5) is a *Cl. parabotulinum equi* type, that is *Cl. botulinum C*.

Although one might expect the toxins of all three rats to belong to one type, there seems to be no reason why they should not differ, as they were caught in different parts of the laboratory grounds. Details of the experimental work carried out with carcasses of rats appear in an article by the writer (1929), shortly to appear, but at present in the press. They were carried out with the idea of throwing some light on the origin of the outbreak of botulism in mules which occurred at this Laboratory in 1924, and from which the strain of *Cl. botulinum*, known as *Cl. parabotulinum equi*, was isolated, later found to be identical with *Cl. botulinum C* of Bengtson (1922) and Graham and Boughton (1923). On testing the two toxins from outbreaks of botulism in horses and donkeys in Johannesburg district, and given in the toxin list as 11 and 628, interesting results were obtained.

TABLE 13.

Toxin.	Antitoxin.	Result.
Cl. botulinum (11), 2 M.L.D.'s.....	*Cl. botulinum* D, 3 c.c.....	Survived.
,, ,, ...	*Cl. parabot. equi* (C), 3 c.c.....	Died.
,, ,, ...	*Cl. botulinum* (rat 6), 3 c.c.....	,,
,, ,, ...	,, (550), 3 c.c.....	,,
,, ,, ...	,, A, 1 c.c.....	,,
,, ,, ...	,, A + B, 1 c.c.....	,,
,, ,, ...	No antitoxin.....	,,

On repeating the test similar results were again obtained with one exception, in that the antitoxin of Rat 6 protected against toxin 11, which may, therefore, be classified as a D type.

TABLE 14.

Toxin.	Antitoxin.	Result.
2 M.L.D.'s *Cl. botulinum* (628).....	3 c.c. *Cl. botulinum D*.....	Survived.
,, ,, ...	3 ,, ,, *C* (*equi*).....	,,
,, ,, ...	3 ,, ,, 554 (D type)..	Died.
,, ,, ...	3 ,, ,, 550 (C type)..	Survived.
,, ,, ...	1 ,, ,, *A*.....	Died.
,, ,, ...	1 ,, ,, *A* + *B*.....	,,
,, ,, ...	No antitoxin.....	,,

The results in this table are not uniform in that one guinea-pig given *Cl. botulinum D* antitoxin survived as well as two with C type antitoxins. A further test was, therefore, carried out with similar results. On carrying out a further test the results were again similar with the exception that the D antitoxin in this case failed to protect, but the control survived as well.

In order to get a better idea of how this toxin should be classified a larger dose of it was used, the results being given in Table 15.

TABLE 15.

Toxin.	Antitoxin.	Result.
5 M.L.D.'s *Cl. botulinum* (628).....	3 c.c. *Cl. botulinum D* (*bovis*).....	Died.
,, ,, ...	3 ,, ,, *C* (*equi*).....	Survived.
,, ,, ...	3 ,, ,, *C* (550).....	,,
,, ,, ...	None.....	Died.

A further series of tests was carried out, the results being given in Table 16.

TABLE 16.

Toxin.	Antitoxin.	Result.
2 M.L.D.'s *Cl. botulinum* (628).....	3 c.c. (*Cl. botulinum*, 554 *D*).....	Died.
,, ,, ...	3 ,, (,, 550).....	Survived.
,, ,, ...	3 ,, (,, *C*).....	,,
,, ,, ...	5 ,, (,, *D bovis*).....	Died.
,, ,, ...	No antitoxin.....	,,

From this and previous tables, therefore, it would seem that *Cl. botulinum* 628 toxin must be classified as a C type. A small quantity of Seddon antitoxin having been made, it was decided to try the effect of it on the 628 toxin. The results, as will be seen from Table 17, were rather unexpected. The antitoxin was made by the use of anatoxin alone and on testing it, 5 c.c. was found to protect against about 20 M.L.D's of *B. parabotulinus* toxin.

The rabbits used for the test were given doses of 3, 5, 7 and 10 c.c. of anatoxin intraperitoneally, at weekly intervals, being bled a week after the last injection.

TABLE 17.

Toxin.	Antitoxin.	Result.
2 M.L.D.'s *Cl. botul.* (628).....	5 c.c. *B. parabot.* (Seddon).....	Survived.
„ „ *D* (*bovis*).....	„ „	Died.
„ „ *C*.....	„ „	„
„ *B. parabot.* (Seddon)....	„ „	Survived.
„ „ „	No antitoxin.....	Died.
„ *Cl. botul. C*, U.S.A.....	„	„

From this table it appears that *Cl. botulinum* 628 is of the *B. parabotulinus* type, that is to say, it is a variant of the C type. This observation is of considerable interest, as it tends to show that there are probably intermediate types of the *C. botulinum* not corresponding exactly to either the A, B, C or D types. Pfenninger's (1924) observation as to the antitoxin against *B. parabotulinus* only neutralizing its own particular toxin, is confirmed.

The toxin of *Cl. botulinum* 628 may, therefore, be considered as a variant of the C type corresponding very closely to the *Cl. botulinum* of Seddon (*B. parabotulinus*).

In a private communication to the writer, Dr. K. F. Meyer, after exhaustive testing of strain 628, has come to the conclusion that it is a B type. This means that the presence of both A and B types has been demonstrated in South African botulinus culture material.

The last three toxins to be typed were as follows:—

(1) Zoo strain (from intestinal contents of wild ducks in Pretoria Zoo).

(2) Bekker strain [from carcass material from cases of botulism (lamsiekte) in sheep at Bredasdorp, Cape Province].

(3) Carlisle strain (from intestinal contents of turkeys in an outbreak of botulism in these birds on a farm near Pretoria).

The first strain was obtained from the caeca of a species of ornamental duck, one of eighteen, which died at Pretoria Zoo after a heavy rainfall. These ducks were all in one small pond, and all showed typical symptoms of botulism. The outbreak is described in an article by the writer (1929), but unfortunately the source of the infection could not be traced. Strains of an organism of the botulinus type were obtained from the caeca of three of these ducks.

The second one referred to as Bekker strain, was of great interest, as it was obtained from an outbreak of lamsiekte (botulism) in sheep. The disease in the Bredasdorp area of the Cape Province is associated

with a definite phosphorus deficiency in the pasture, causing the sheep to show a marked tendency to develop osteophagia. There is an unusual brittleness of bone shown by the sheep in this area and fractures of ribs or long bones are very common. Lamsiekte in sheep is uncommon in the Union of South Africa though occasionally seen in goats. Recently, Sigwart (1929) has published a paper on "Lamsiekte in Sheep" in South West Africa. The symptoms described leave no doubt as to the disease being true lamsiekte as seen in cattle. It occurs chiefly in Africander sheep, but may also be seen in the merino. Carcass material from the farms where the disease occurs has not yet been tested for botulinus toxins, but material will shortly be available for this purpose.

The third strain (Carlisle) was obtained from the caecal contents of a turkey, one of fifteen young adult birds which died on a farm near Pretoria. They were all affected at the same time and showed definite symptoms of botulism. The source of the infection could not be traced, but the turkeys were running free and the owner could not be certain what they had been eating.

In the first table the three strains were tested with a combined *Cl. botulinum A* and *B* antitoxin.

TABLE 18.

Toxin.	Antitoxin.	Result.
2 M.L.D.'s *Cl. botulinum* (Carlisle)...	1 c.c. botulinum A + B.....	Died.
2 ,, ,, (Bekker)...	,, ,, ...	,,
2 ,, ,, (Zoo).....	,, ,, ...	,,
5 ,, ,, *A*.....	,, ,, ...	Survived.
5 ,, ,, *B*.....	,, ,, ...	,,
5 ,, ,, *A*.....	No antitoxin.....	Died.
5 ,, ,, *B*.....	,, ...	,,

The three strains were, therefore, not *Cl. botulinum A* or *B* types.

Further tests were then carried out using three different antitoxins, *Cl. botulinum D*, *Cl. botulinum C* (*equi* type) and *Cl. botulinum* 550, also an *equi* type. The results were uniform, as will be seen from the following three tables.

TABLE 19.

Toxin.	Antitoxin.	Result.
5 M.L.D.'s *Cl. botulinum* (Carlisle)...	2 c.c. *Cl. botulinum C* (*equi*).....	Survived.
5 ,, ,, (Bekker)...	,, ,, ...	Died.
5 ,, ,, (Zoo).....	,, ,, ...	Survived.
5 ,, ,, (*equi*).....	,, ,, ...	,,
2 ,, ,, ,, ...	No antitoxin.....	Died.

The turkey and zoo strains therefore corresponded to the *Cl. botulinum C* type.

TABLE 20.

Toxin.	Antitoxin.	Result.
5 M.L.D.'s *Cl. botulinum* (Carlisle)...	3 c.c. *Cl. botulinum* 550 (C type)..	Survived.
5 „ „ (Bekker)...	„ „ „ ..	Died.
5 „ „ (Zoo).....	„ „ „ ..	Survived.
5 „ „ 550 (C)....	„ „ „ ..	„
2 „ „ 334 (D)....	„ „ „ ..	Died.

The results in this table confirm those in the previous one, the antitoxin being of the same type.

TABLE 21.

Toxin.	Antitoxin.	Result.
5 M.L.D.'s *C. botulinum* (Carlisle)...	3 c.c. *Cl. botulinum D*.....	Died.
5 „ „ (Bekker)...	„ „	Survived.
5 „ „ (Zoo).....	„ „	Died.
5 „ „ (334) *D*....	„ „	Survived.
2 „ „ (334) *D*....	No antitoxin.....	Died.

The strain from material from cases of lamsiekte in sheep, therefore, belongs to the *Cl. botulinum D* type. This is a very interesting fact in view of the distribution of the disease in cattle and sheep.

The three strains just described may, therefore, be classified as follows:—

(1) *Cl. botulinum* (Zoo strain, from birds) is a C type.
(2) *Cl. botulinum* (Bekker strain, from sheep) is a D type.
(3) *Cl. botulinum* (Carlisle strain, from turkey) is a C type.

It may be of interest to give a composite table showing the results obtained with toxin-antitoxin tests on all the strains used:

TABLE SUMMARIZING TOXIN ANTITOXIN TESTS.

Cl. botulinum D. (*Cl. parabot. bovis.*)	*Cl. botulinum C.* (*Cl. parabot. equi.*)	*Cl. botulinum C.* (Graham, U.S.A.)	*B. parabotulinus.* (Seddon.)	*Cl. botulinum.* (Rat 3.)	*Cl. botulinum.* (Rat 5.)	*Cl. botulinum* 544. (Rat 6.)	*Cl. botulinum* 333.
D (bovis) = + D (bovis) = + A = − B = − A + B = − C (equi)	C (equi) = + C = + D (bovis) = − A + B = −	D (bovis) = − C = + C (equi) = + A + B = − B. parabot. = −	D (bovis) = − A + B = − C = + B. parabot = +	bovis (rat 6) = +	C (335A) = + C (equi) = − D (bovis) = − A = − B = − D (Rat 6) = −	D bovis = + D (Rat 6) = + D (Rat 6) = + C (335 A) = −	C (equi) = + D (bovis) = − Rat 6 (bovis) —
D type.	*C type.*	*C type.*	*C type.*	*D type.*	*Probably C type.*	*D type.*	*C type.*

Cl. botulinum. 334	*Cl. botulinum.* 335	*Cl. botulinum.* 335 (A)	*Cl. botulinum* 628. (Horses in Johannesburg.)	*Cl. botulinum* 11. (Donkeys, Johanuesburg.)	*Cl. botulinum.* (Turkey Strain.)	*Cl. botulinum.* (Bekker, sheep lamsiekte.)	*Cl. botulinum.* (Zoo bird strain.)
C (equi) = − D (bovis) = + D (Rat 6) = + C (335A) = −	C (equi) = − D (bovis) = + C (equi) = − D (bovis) = +	C (equi) = ± C (equi) = + D (bovis) = − D (bovis) = −	D (bovis) = − D (bovis) = − C (equi) = + C (335A) = + A + B = − B. *parabot.* = + (Seddon)	D (bovis) = + C (equi) = − D (Rat 6) = + C (335A) = − A = − A + B = −	C (equi) = + C (equi) = + C (equi) = + D (bovis) = − D (bovis) = − D (bovis) = − A + B = −	335 (A) = ± C (equi) = − C (equi) = − C (equi) = − D (bovis) = + D (bovis) = + D (bovis) = + A + B = −	C (equi) = + C (equi) = + C (equi) = + D (bovis) = − D (bovis) = ± A + B = −
D type.	D type.	C type.	C type.	D type.	C type.	D type.	C type.

The toxin is given above the column and the antitoxin in the column. Where an antitoxin appears more than once in a column it means the test was repeated. Result appears under the column.

\+ = neutralised.
− = not neutralised.
± = doubtful result.

There is no doubt that classification of types of *Cl. botulinum* toxin by cross toxin-antitoxin tests forms the best means for the purpose at present available. With impure cultures one may sometimes get toxins which are difficult to classify absolutely definitely, but even with them one can generally accomplish this satisfactorily.

The bearing of this work on the question of immunization against botulism in animals, more particularly cattle, sheep and goats, is of great interest. It shows that any such immunization, if it were ever considered advisable, would have to be done with polyvalent antitoxins in the case of passive and anatoxins in that of active immunization.

GENERAL CONCLUSIONS.

It is clear from the work carried out by many investigators, including the writer, that apart from the A and B types of the *Clostridium botulinum*, one has to deal with at least two other types in nature, namely the C and D types.

All the types dealt with by the writer can be classified either under the C or the D one, as judged by comparative toxin-antitoxin tests. The position of the *B. parabotulinus* of Seddon is still somewhat uncertain though it is definitely related to the C type.

Meyer (K. F.), in a private communication to the writer mentions that he obtained an organism of the *Cl. botulinum A* type from a culture sent to him by the writer. This is the only record of the occurrence of this particular type in South Africa.

It would appear that the organism described by Theiler and the writer as "*Clostridium parabotulinum equi*" must definitely be regarded as identical with the C type of Bengtson and, that of Graham and Boughton as judged by toxin-antitoxin tests. There is no doubt, however, that it is very much more toxic for horses than the American types. The organism described as "*Clostridium parabotulinum bovis*" must now definitely be classified as "*Cl. botulinum D*" (Theiler and Robinson) as suggested by Meyer and Gunnison (1929). It must, however, be emphasized that organisms which belong to the C type exist on "lamsiekte" farms and therefore, "lamsiekte" can no longer be considered as due to one particular toxin.

In the foregoing pages the writer has attempted to summarize our knowledge of the organisms of the *Cl. botulinum C* and *D* types at the present date, and give a survey of the known facts in connection with them.

The comparison by morphological and cultural characteristics does not point to any special features which would be of value in distinguishing the bacteria classified under these two types, in fact, one is struck by their close resemblance in almost every particular.

Comparative toxicity tests cannot be regarded as of much value in differentiating the types dealt with. There are marked differences in toxicity for certain animal species shown by different types, but it is quite possible, judging from our knowledge of exo-toxins in general, that the same type of the *Clostridium botulinum* isolated from different sources might have a very variable toxicity.

Comparative agglutination tests have given very interesting results, and are of undoubted value in indicating the relationships of various *Clostridium botulinum* types. The group relationships which exist are very well brought out by these tests.

Finally, comparisons by toxin-antitoxins tests were carried out and proved by far the most valuable means of classifying these organisms. One particular advantage of them is that one can attempt the classification of toxins from impure cultures with a fair degree of success.

The writer is well aware of the defects which still exist in our knowledge of the bacteria of the clostridium C and D types, but recognizes the value of from time to time reviewing our knowledge in connection with a particular subject or part of a subject, and it was with this idea that the present article was written.

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A Strain of *Mycobacterium tuberculosis* from the Giraffe and further Observations of *M. tuberculosis* from the Koodoo and the Duiker.

By G. MARTINAGLIA, M.Sc., V.S., D.V.Sc., Veterinary Research Officer, Onderstepoort.

A GIRAFFE (*Giraffa cameleopardalis*) in the Johannesburg Zoo which had been ailing for some time, died on 13.8.29. The public had taken considerable interest in this animal's illness and, in the local papers, it was reported to be suffering from gastric disturbance. A post-mortem was held by Mr. Kirkpatrick, M.R.C.V.S., Assistant Superintendent of the Abattoirs, and a diagnosis of tuberculosis of the lungs was made. A piece of the lung was thereupon sent to Onderstepoort in order to ascertain whether the disease was of human or bovine origin.

Microscopical examination of material from the lesions showed a fair number of acid fast organisms to the field, some having a beaded appearance. Directly after treatment with a 6 per cent. sodium hydroxide solution, for half an hour, cultures were made. An excised bit of the diseased lung was treated by the above method and after centrifugalizing and washing, the sediment was injected intraperitoneally into two guinea-pigs, and two rabbits. After 6 weeks the guinea-pigs and rabbits were chloroformed; neither of the rabbits showed lesions of tuberculosis. The guinea-pigs on the other hand showed tuberculosis of the liver, spleen, omentum, peritoneum and lungs. From the omental nodules, cultures were made, directly on glycerinated blood agar. On the 18th day after seeding, typical colonies of *M. tuberculosis* were noticed. A platinum loopful (4 mm. diameter) of a month old growth was added to 10 c.c. of normal saline solution and well shaken. There were on an average 50 bacilli to a field. With this emulsion two fowls and two rabbits were injected, each receiving 1 c.c. intravenously. Four months after the injection one rabbit died from tuberculosis of the lungs; there were present, numerous acid fast organisms. No other macroscopic lesions were noted. The second rabbit died three weeks later, in fair condition; there was a slight peritonitis but no acid fast organisms could be demonstrated. One fowl died two months after injection but no lesions of tuberculsois were observed. The remaining one was chloroformed five months after injection. On autopsy, it was found to be in good condition and no signs of tuberculosis were noted.

A calf, three months old, was injected subcutaneously behind the shoulder, as advocated by Kossel, Weber and Heus (1905). Subsequently very little swelling was noticed at the point of injection. After four months the calf was killed and autopsied. It was in excellent condition and no sign of tuberculosis was noted. According to animal inoculation results this strain appears to conform to the human type of *Mycobacterium tuberculosis*.

In a previous communication on tuberculosis in wild buck living under natural conditions, Paine and Martinaglia (1929), demonstrated that the strains isolated were of the bovine type. After publication it was decided that two cattle be inoculated, one with the Koodoo (*Strepsiceros strepsiceros*) and the other with the Cape duiker (*Sylvicapra grimmi*) strain. The object was to make controls for tuberculin testing and also to ascertain whether these buck strains are pathogenic for cattle.

The two cows selected were negative to the tuberculin test and, like the rest of the herd, in the best of condition.

On 9.8.28 Cow No. 727 received 2.5 c.c. saline emulsion made from a blood agar slant of four weeks' growth (Koodoo strain). A firm swelling developed slowly until it reached the size of a man's fist. When the animal was slaughtered 17 months after injection, she was very emaciated, and on autopsy manifested extensive generalized tuberculosis practically every organ being involved.

The Cape duiker strain was injected into Cow No. 1307 at the same time as the Koodoo strain. Two platinum loopfuls from a four weeks' old growth on blood agar were added to 10 c.c. of normal saline solution and well shaken. One c.c. of the emulsion was injected on the side of the neck under the skin. A slight lump developed and disappeared after 6 months. Nineteen months after injection this cow died from acute anaplasmosis. On autopsy the internal organs showed no macroscopic lesions but the udder displayed typical caseous abscesses in which acid fast organisms could be demonstrated. During the period under observation these animals were tested with several tuberculins (including one prepared from the Koodoo strain) and always gave marked reactions. The first test was made six weeks after the injection with tubercle bacilli.

It will be noted from these cases that wild animals are subject to both strains of *Mycobacterium tuberculosis*.

As a possible menace to the health of the fauna in our national game reserves, tuberculosis must be considered. The advice of a veterinary epizootologist should be constantly available to those in charge of these reserves, in order to control the introduction of infectious diseases.

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Cow infected with *Mycobacterium tuberculosis* from the Koodoo and tested for tuberculosis by the intra-orbital injection of Tuberculin.
Note the swelling below the left eye.

A Note on the Addition of Glycerine, as a Preservative, for Anthrax Spore Vaccine.

By J. G. BEKKER, B.V.Sc., Veterinary Research Officer, Armoedsvlakte Experimental Station.

Glycerine is often used as a preserving agent for vaccines. In this Laboratory Anthrax spore vaccine is contained in a solution of glycerine and normal saline. This solution is made up by adding 60 per cent. glycerine to 40 per cent. normal saline (by volume).

The glycerine acts beneficially as it preserves the Anthrax spores, prevents any further vegetation of these and probably destroys any vegetative forms which might be present. Contamination of the vaccine by other organisms is either prevented or the organisms prevented from growing and thus making the vaccine unsuitable for safe use.

Viljoen and his co-workers, in their report on "Anthrax in South Africa," give no reasons why the glycerine should be added to an extent of 60 per cent. Keeping in mind the value of glycerine to the vaccine, the following cultural observations were made to determine whether it is possible to considerably reduce the amount of glycerine, which should be added to the spore emulsion, without reducing its effectiveness for the stated purposes:—

EXPERIMENT I.

Object.—To determine the concentration of glycerine which will effectively prohibit the vegetation and growth of Anthrax vaccine spores.

Methods.—I. To ordinary nutrient broth and nutrient agar various amounts (by volume) of glycerine is added.

II. As an inoculum spore vaccine emulsion Batch 87, (Goat Vaccine) prepared from Strain 5.b. Batch 53 is used.

III. Tubes kept in incubator at 37° C.

Per cent. Glycerine.	Ordinary Nutrient Agar.								Ordinary Nutrient Broth.							
	0%	5%	10%	20%	30%	40%	50%	60%	0%	5%	10%	20%	30%	40%	50%	60%
21/1/27	Tubes Inoculated.								Tubes Inoculated.							
22/1/27	††	††	†	sl.	sl.	—	—	—	††	††	††	†	sl.	—	—	—
23/1/27	††	††	†	sl.	sl.	—	—	—	††	††	††	†	sl.	—	—	—
24/1/27	††	††	†	sl.	sl.	—	—	—	††	††	††	†	sl.	—	—	—
25/1/27	††	††	†	sl.	sl.	—	—	—	††	††	††	†	sl.	—	—	—

Note.— †† = Profuse growth.
† = Fair growth.
sl. = Slight growth.
— = No growth.

EXPERIMENT II.

Object.—To determine the concentration of glycerine which will effectively prohibit the vegetation and growth of:—

(*a*) Anthrax vaccine spores.

(*b*) *B. subtilis* spores.

Methods.—I. As in experiment I.

II. As an inoculum the following is mixed:—

(*a*) Spore emulsion of Anthrax vaccine Batch 88, prepared from 3 F.B. Batch 68 (cattle vaccine).

(*b*) Emulsion of *B. subtilis* spores.

III. Tubes kept at 37° C.

Per cent. Glycerine.		Ordinary Nutrient Agar.								Ordinary Nutrient Broth.							
		0%	5%	10%	20%	30%	40%	50%	60%	0%	5%	10%	20%	30%	40%	50%	60%
Anthrax.... / *B. Subtilis.*	21/1/27	Tubes Inoculated.								Tubes Inoculated.							
Anthrax....	22/1/27				†	sl.	—	—	—	††	††	††	sl.	sl.	—	—	—
B. Subtilis..		††	††	††	—	—	—	—	—	††	††	††	—	—	—	—	—
Anthrax....	23/1/27				†	sl.	—	—	—	††	††	††	†	sl.	—	—	—
B. Subtilis..		††	††	††	—	—	—	—	—	††	††	††	—	—	—	—	—
Anthrax....	24/1/27				†	sl.	—	—	—	††	††	††	†	sl.	—	—	—
B. Subtilis..		††	††	††	—	—	—	—	—	††	††	††	—	—	—	—	—
Anthrax....	25/1/27				†	sl.	—	—	—	††	††	††	†	sl.	—	—	—
B. Subtilis..		††	††	††	—	—	—	—	—	††	††	††	—	—	—	—	—

NOTE.— †† = Profuse growth.
† = Fair growth.
sl. = Slight growth.
— = No growth.

DISCUSSION.

It will be seen from the results of Experiment I that 40 per cent. glycerine completely stops all growth of Anthrax.

In Experiment II the same is seen. Here it will also be seen that 20 per cent. glycerine prohibits and stops all growth of *B. subtilis*. The *Bacillus subtilis* was specially selected, as its appearance on agar and in bouillon cultures can easily be distinguished from Anthrax, and because it is an organism which grows readily on a wide range of media, it is also a very common organism in nature and frequently vaccines are contaminated with it. The effect of the glycerine on other than vaccine Anthrax could be determined.

CONCLUSION.

It is not necessary to use glycerine to the extent of 60 per cent. for preserving Anthrax spore vaccine, a 40 per cent. concentration (by volume) will suffice.

Section IV.

Parasitology.

G. A. H. Bedford — New Genera and Species of Mallophaga from South African Hosts.

H. O. Mönnig ... — Studies on the Bionomics of the Free-living Stages of Trichostrongylus spp. and other Parasitic Nematodes.

H. O. Mönnig ... — A Note on the Preservation of Engorged Female Ticks.

P. L. le Roux ... — A Spirurid (*Streptopharagus geosciuri* sp. nov.) from the Stomach of the Cape Ground Squirrel (*Geosciurus capensis*).

P. L. le Roux ... — The Generic Position of *Oxyuris polyoon* von Linstow, 1909, in the Sub-Family *Oxyurinae*, Hall, 1916.

P. L. le Roux ... — An Acuaria (*Acuaria Martinagliai* sp. nov.) from a South African Weaver (*Hyphantornis* sp.).

P. L. le Roux ... — A New Nematode (*Rictularia aethechini* sp. nov.), a *Physaloptera* and an *Acanthocephala* from the Hedgehog (*Aethechinus frontalis*).

P. L. le Roux ... — On two New Helminths from the Abomasum of the Bushbuck in Zululand, Natal.

P. L. le Roux ... — A Preliminary Communication on the Life Cycle of *Cotylophoron cotylophorum* and its Pathogenicity for Sheep and Cattle.

J. L. Papert ... — Tsetse-fly Survey of Zululand and Surrounding Territories.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

New Genera and Species of Mallophaga from South African Hosts.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

SUPER-FAMILY *AMBLYCERA* KELLOGG.

FAMILY MENOPONIDAE MJÖBERG.

Genus Allomenopon nov.

Species of medium size, pale in colour.

Head triangular, slightly more than $\frac{1}{3}$ wider than long. On each side of the forehead there is a slit above the eyes, which are situated a short distance from the temporal margins. Pharyngeal sclerite and glands absent. Antennary fossa deep. Antennae four-jointed, the third segment constricted at the base.

Prothorax large and broad, with acute wings. *Meso-* and *metanotum* not separated by a suture, the lateral margins strongly divergent, the posterior margin nearly straight.

Legs normal, the anterior femora slightly shorter than the mid femora, the posterior pair slightly longer than the mid, with a patch of about forty short hairs on the venter.

Abdomen broad, rounded posteriorly, with the tergites, sternites, and pleural plates very weakly chitinized. Tergites with a single row of hairs on their posterior margins. Sternites with three or four irregular rows of short hairs, the hairs more numerous and forming a tuft on each side of the fourth and fifth segments, and to a lesser extent on the sixth sternite. Spiracles present on the lateral margins of the third to eighth segments. Male genitalia with the basal plate short and broad; parameres slender; endomeral plates with backward-projecting curved hooks on each side. Species found on Bucerotidae (hornbills).

Genotype: *Menopon bucerotes* Kellogg.

Menopon lophocerus Bedford (7th and 8th Rep., Dir. Vet. Res., Union of S.A., 1920 p.p. 717-718, Pl. 1, f. 1, Pl. 3, f. 1) found on *Lophoceros epirhinus*, *Tockus erythrorhynchus rufirostris*, and *Xanthorhynchus leucomelas*, must also be included in this genus. *M. bucerotis* Kellogg (Schwed. Exp. Kilimandjaro, 1910, p. 54, Pl. 7, f. 12) occurs on *Baryrhynchus cristatus* and *Bycanistes bucinator*. The male genitalia of both species have been figured by Bedford (1920, Pl. 3, f. 1, 2).

Genus Psittacomenopon nov.

Species medium-sized, dark.

Head about $\frac{1}{3}$ wider at the temples than long. Forehead rounded; lateral margins with a slit in front of the eyes which are situated on the margins of the temples. On each side near the base of the slit there is a blotch. Mandibles large, bidentate in the female, but only one with two teeth in the male. Pharyngeal sclerite and glands well developed. Antennary fossa deep. Antennae four-jointed, the second segment with a large anterior expansion, the third constricted at the base.

Prothorax large and broad, with moderately acute wings.

Mesothorax very short, the notum separated from the metanotum by a suture.

Metathorax very large, with the lateral margins strongly divergent, the posterior margin projecting backwards on to the abdomen in the female, almost straight in the male.

Legs normal; the posterior femora with a series of three combs on the venter.

Abdomen of female elongate and slender, pointed posteriorly. The first tergite considerably longer than the others, except the apical one, projecting backwards on to the second segment, with several chitinous transverse lines on each side in front. The tergites and sternites with well-developed transverse plates; the plates on the third to eighth tergites divided longitudinally into three areas, and on the apical tergite the plate is divided in the middle. Tergites with two transverse rows of hairs. Sternites with numerous short hairs, and on the third segment there are two combs on each side. Apical sternite with two plates fringed with hairs. The abdomen of the male is shorter and narrower than that of the female, the first segment is slightly longer than the second, but does not project backwards on to the second, and there are no transverse lines. Genitalia with the basal plate long and narrow; parameres long, narrow, and slightly curved.

Species found on Psittacidae (parrots).

Genotype: *Menopon poicephalum* Bedford.

In this genus must also be included: *Menopon impar* Piaget and *Menopon scalaris* Piaget. Unfortunately we have not been able to make either of these species the type of the genus, because we have not seen specimens and the males are still unknown. Both *scalaris* and *poicephalum* were described as varieties of *impar*, but they are quite distinct from that species. *P. poicephalum* was described from a male and female taken off a *Poicephalus meyeri* (Meyer's parrot), and we have recently received specimens from Mr. Austin Roberts taken off *Poicephalus robustus* Gm. (Cape parrot) at Haenertsburg, Transvaal, on the 14th January, 1930.

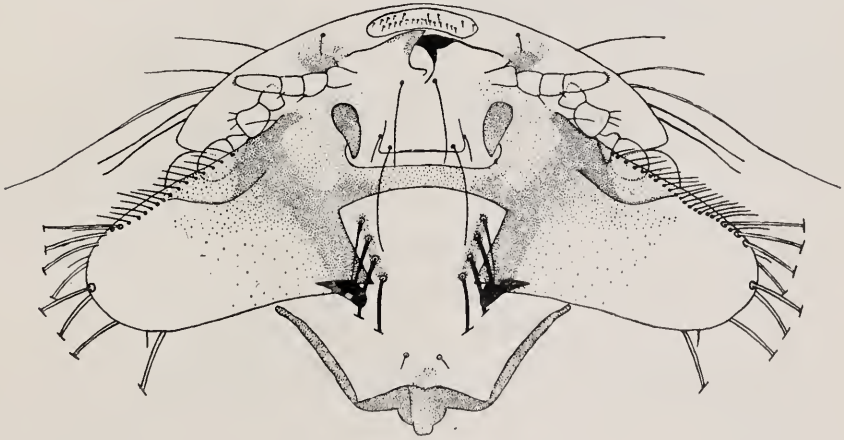
Genus Menacanthus Neumann.

Menacanthus corvus nov. sp.

(Figs. 1, 2, and 3.)

Largish species, pale in colour with conspicuous dark brown bands and markings.

Female.—*Head* (fig. 1) very slightly more than twice as wide as long. Forehead rounded with a slit on the lateral margins in front of the temporal angles. Eyes large, situated at the temporal angles; on the inner margin of each there is a hair. Antennae four-jointed; the third joint constricted at the base. Temples projecting, with eight long hairs and several short ones. On the dorsum of the forehead there are one long and two short hairs situated close together on each side between the meson and lateral margin. Occipital margin with six long hairs. On the venter there is a large backward projecting spine on each side beneath the base of the palpus, and below these spines a median transverse bar. On the gular region there is a narrow longitudinal chitinous band with four long hairs on each side.



G. A. H. B. del. Fig. 1.

Menacanthus corvus nov. sp., venter of head and sternal plate of female.

Thorax: *Pronotum* more than twice as wide as long, with the antero-lateral angles rounded. Beneath the transverse bar there are two minute hairs, and one at each end of it. On each lateral margin there is a minute hair and a long one at the angle, and two more long ones beneath them, these being widely separated. On the posterior margin there are eight long hairs. *Mesonotum* short, with two short hairs on each side, the outer pair near the postero-lateral angles. *Metanotum* with the lateral margins strongly divergent, on the posterior margin there is a row of about twenty long hairs, and on each side four to five short hairs, one above the other, close to the

lateral band. *Prosternum* with plate as shown in fig. 1. On the *mesosternum* there is a small median chitinized plate bearing several short hairs and a similar plate on the *metasternum*, the latter being wider than long. On each side between the mid and hind femora there is a small chitinized area with three or four minute hairs.

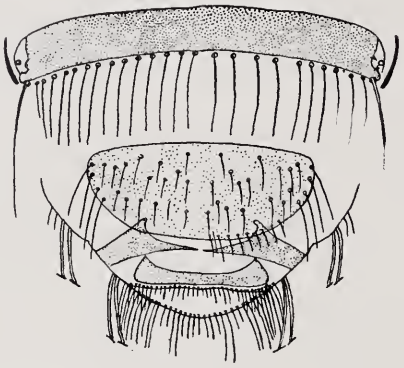


FIG. 2.

Menacanthus corvus nov. sp., seventh tergite and apical sternites.

G. A. H. B. del.

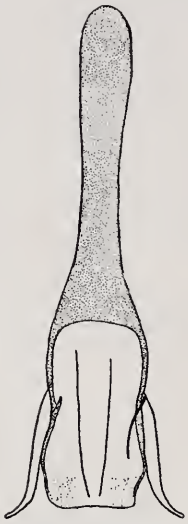


FIG. 3.

Menacanthus corvus nov. sp., male genitalia.

Legs normal, the hind femora with a patch of about twenty-four hairs on the venter.

Abdomen widest at the fourth segment. Tergites with strongly chitinized transverse bands and with a row of long hairs on the posterior margins (fig. 2A). Sternites one to seven each, with a dark transverse band and numerous short hairs, which are somewhat clustered at the lateral margins. Apical sternites as in fig. 2B. Pleurites well developed with short hairs and long ones on their posterior margins. Spiracles small, present on the third to eighth segments.

MALE very similar to the female. Genitalia as in fig. 3.

	Measurements.			
	Female.		Male.	
	Length.	Width.	Length.	Width.
	Mm.	Mm.	Mm.	Mm.
Head	0·35	0·77	0·36	0·72
Prothorax	0·24	0·57	0·2	0·53
Meso-meta-thorax	0·21	0·71	0·16	0·62
Abdomen	1·79	1·14	1·51	0·9
TOTAL	2·59		2·23	

Holotype: a female from *Corvus albus* Müll.

Described from numerous females and males taken off two specimens of *Corvus albus* Müll. (pied crow), Aliwal North, C.P., December, 1929, and January, 1930 (Coll. G. Martinaglia and P. L. le Roux); also females and males taken off *Heterocorax capensis* Lcht. (black crow) at Onderstepoort, on the 26th January, 1921 (Coll. G.A.H.B.).

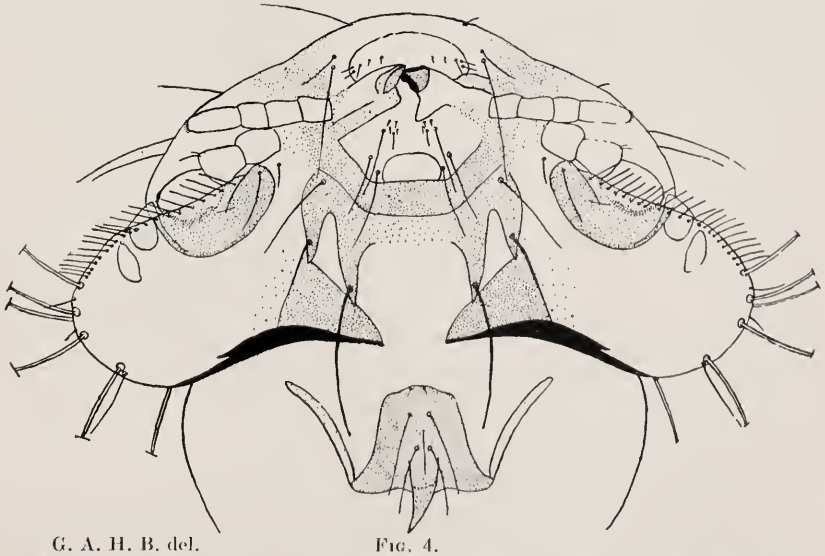
Genus Machaerilaemus Harrison.

Machaerilaemus urocolius nov. sp.

(Figure 4.)

A robust form, pale in colour, with yellowish-brown to brown bands and markings.

Female.—*Head* (fig. 4) very slightly more than twice as broad at the temples as long. Forehead rounded. Eyes well developed, hairless. Temples rounded, with six long hairs and several short ones. On the dorsum of the forehead there are a long and two short hairs on each side between the meson and lateral margin. Occiput with six long hairs on the margin. On the ventral surface there is a transverse chitinized bar behind the mandibles which projects forwards on each side to the anterior margin. Gular plate considerably wider than long with two pairs of backward-projecting spinose processes, the front pair each with two long hairs and the posterior pair each with one long hair. On each side of the gular region behind there is a triangular chitinized area. Antennae three-segmented. Palpi with the apical segment the longest, the second segment only slightly longer than either the first or third, which are sub-equal.



G. A. H. B. del. Fig. 4.

Machaerilaemus urocolius nov. sp., venter of head and sternal plate of female.

Thorax: *Pronotum* very slightly more than twice as wide as long, lateral margins rounded, each with a long hair in front, three short ones above it and a short one below it, and beneath the latter there are two more short ones, one above the other. On the posterior margin there are ten long hairs and four short ones. Plate on sternum as shown in fig. 4. *Mesonotum* not separated from the metanotum by a suture, the former with a short hair on each side near the antero-lateral margin and two more beneath it. *Metanotum* with numerous long and short hairs on the posterior margin, and three or four on each side above one another close to the lateral band. On the mesosternum there is a very small indistinct plate with two short hairs, and a larger one on the metasternum with several short hairs.

Legs: Hind femora with a patch of about thirty hairs on the venter; mid and hind tibiae each with three short, strong hairs at the apex.

Abdomen slightly broader than long; the tergites, except the last, each with a pale brown transverse band and a series of long hairs on the posterior margins, at the sides there are one or two short hairs between the long ones. Sternites also with pale brown transverse bands and with three irregular rows of hairs, the hairs being slightly more numerous at the sides on the fourth to seventh sternites. The borders of the vulva are closely beset with a row of fine hairs. Pleurites pale brown with several hairs in the middle, and a row of long and short hairs on their posterior margins.

Male.—The male resembles the female, except that there are two transverse bands on the eighth sternite. Genitalia weakly developed, especially the basal plate, which is short and not expanded apically, articulating with a pair of very narrow parameres which curve inwards.

	Measurements.			
	Female.		Male.	
	Length.	Width.	Length.	Width.
	Mm.	Mm.	Mm.	Mm.
Head	0·36	0·74	0·33	0·65
Prothorax	0·25	0·54	0·22	0·45
Meso- and meta-thorax	0·26	0·87	0·18	0·67
Abdomen	1·36	1·41	1·02	1·03
Total	2·23		1·75	

Holotype: a female.

Described from a female and male collected by the writer off *Urocolius indicus transvaalensis* Roberts (red-faced coly) at Ntabanana, Zululand, on the 27th November, 1922; also a female and male taken from the same host at Onderstepoort.

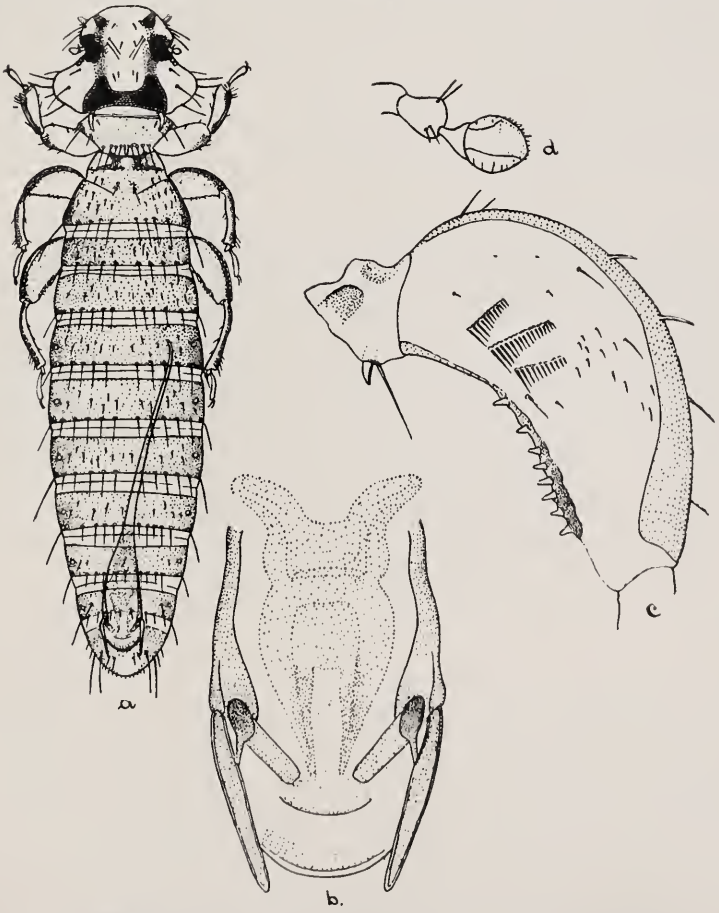
This species can easily be recognized from the other three known species by the presence of the four spines on the gular plate. Both *M. plocci* Bedford and *M. latifrons* Harrison possess only two spines on the gular plate.

Genus Colpocephalum Nitzsch.

Colpocephalum ferrisi nov. sp.

[Figs. 5 (*a*)-(*d*).]

Male.—*Head* wider than long, widest behind the eyes. Forehead rounded in front; lateral margins with a deep notch just in front of the eyes, which are small. On each side of the head there are



C. G. Walker, del. Fig. 5.

Colpocephalum ferrisi nov. sp., (*a*) male; (*b*) male genitalia; (*c*) venter of hind femur; (*d*) antenna.

three large blotches, one on the forehead, a larger one at the base of the notch, and a still larger one on the posterior margins, the median blotches being joined to both the anterior and posterior blotches by slightly paler bands, and the two on the occipital areas are connected by a dark transverse band. Similar markings also present on the venter. Labial palpi slender, extending beyond lateral margin of head. Antennae four-jointed, the third joint stalked at the base, the fourth rounded. Pharyngeal sclerite and glands present. On the venter behind the mouth-parts there is a small transverse plate, and a longitudinal row of six or seven hairs on each side of the meson.

Prothorax with acute wings; at each lateral angle there is a short and long hair, and another short hair on the lateral margin beneath these; on the posterior margin there are eight long hairs and a minute one on each side above the two outer ones; beneath the transverse bar there is a row of four minute hairs. *Mesothorax* short with a narrow transverse chitinous band on the notum. *Metathorax* with the lateral margins strongly divergent; posterior margin almost straight; notum with a few short hairs and a row of longer ones on the posterior margin. Sternal plate triangular, weakly chitinized, with a few short hairs.

Legs: Posterior trochanters with a small spine on their posterior margins. Mid femora with three to five small spines on the posterior margin; hind femora with four rows of combs on the venter, the first row consisting of only two or three minute bristles, and with a row of eight or nine small spines on the posterior margin.

Abdomen elongated and slender, the tergites more strongly chitinized than the sternites, the former sparcely covered with short hairs and a row of longer ones on the posterior margins. Sternites also sparsely covered with short hairs and with two series of combs at the latero-posterior angles of the third sternite. Apical sternite with six long hairs on the posterior margin. Spiracles very small, present on the lateral margins of the third to eighth tergites. Pleurites small, darker than the tergites, with short hairs. Genitalia with the basal plate long and slender; parameres present, with a plate between them which is rounded posteriorly.

	Measurements.	
	Length.	Width.
	Mm.	Mm.
Head	0·35	0·54
Prothorax	0·16	0·36
Meso- and meta-thorax	0·24	0·47
Abdomen	1·65	0·56
TOTAL	2·40	

Described from three males taken off *Melanopelargus niger* Linné (black stork) at Nomgas, S.W.A., in January, 1919.

This species is allied to *C. zebra* Nitzsch, the type of the genus, which has recently been described and figured by Ferris in *Parasitology*, XVI, pp. 59-61, fig. 2, 1924. It can be distinguished from it by its more elongated form, the presence of a band connecting together the anterior and median blotches on the head, the small spines on the posterior margins of the mid and hind femora, and by the genitalia. In *zebra* the genitalia possess a flat serrated plate at the apex of the basal plate which is absent in *ferrisi*, and there are no parameres.

Genus Eucolpocephalum nov.

Head almost twice as wide as long; forehead rounded, lateral margin with a distinct notch in front of the eye; temples rounded. Blotches absent, except for a small brown one on each side of the head at the base of each notch.

Prothorax winged. Mesonotum small, not separated from the metanotum. Plate on prosternum as shown in fig. 6; mesosternal plate triangular with short hairs; metasternal plate four-sided with numerous short hairs; wider in front than behind; anterior margin convex.

Legs: hind femora with a patch of short hairs on the venter.

Abdomen with a narrow transverse chitinous bar on each segment except the last, those on the fourth to eighth segments indistinct, especially in the middle of the abdomen. Tergites and sternites with lightly chitinous transverse bands, the former being wider than the latter and possess one row of hairs on their posterior margins. Sternites covered with short hairs; on the fourth and fifth sternites the hairs are slightly more numerous at the sides. Spiracles present on the lateral margins of the third to eighth segments. Pleurites distinct. Male genitalia with the basal plate short and broad; parameres elongated and narrow, curved inwards.

Genotype: Eucolpocephalum robustum nov. sp.

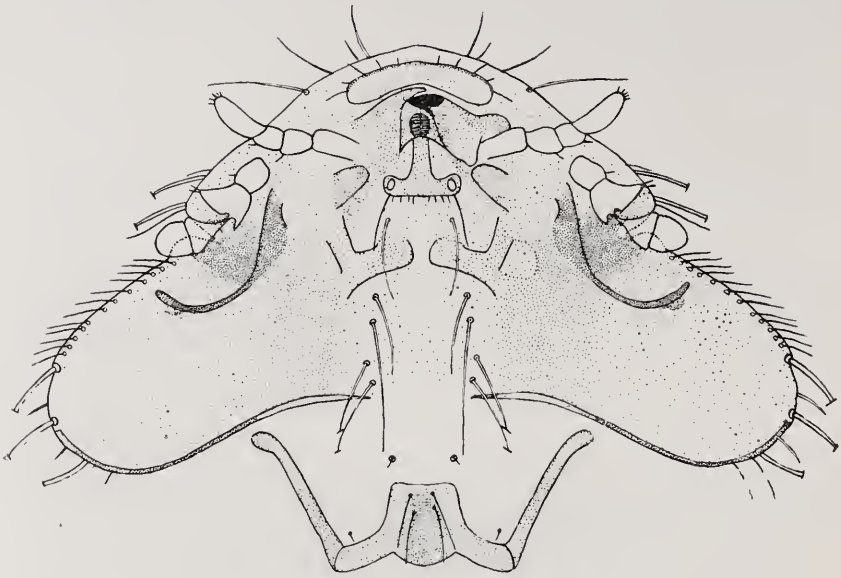
Eucolpocephalum robustum nov. sp.

(Fig. 6.)

A robust form, yellowish-brown in colour.

Female.—*Head*: On the margin of the forehead there are eight hairs on each side, four being situated just above the lateral notch. On the dorsal surface there is a transverse row of four longish hairs between the notches, and above the outer ones there is a long hair and two short ones above it; behind the anterior margin there are two short admedian hairs and four longer ones below them; on the temples there are six long hairs and several short ones; a short hair close to each eye, and five or six long hairs on the posterior margin. All the long hairs arise from large pale pustules. On the venter there are five

short hairs on each side of the meson in front of the mandibles, two longitudinal rows of three short hairs behind the mandibles, and four longer ones on each side on the gular region. Palpi long, projecting beyond lateral margins of head. Antennae four-jointed, the second joint with a marked anterior expansion, the third constricted at the base.



G. A. H. B. del. FIG. 6.

Eucolpocephalum robustum nov. sp., venter of head and sternal plate of female.

Thorax: Pronotum with one or two short hairs at the lateral angle, and two long ones on the margin beneath them; on the posterior margin there are ten long hairs and one short one on each side between the outer pair. Below the transverse bar there are four hairs, the median pair being minute. Metanotum with a transverse row of four hairs in front, beneath the outer ones a short and minute hair on each side, and a row of long and short hairs on the posterior margin.

Abdomen widest at the fourth and fifth segments. Tergites i to vii with a row of long and short hairs on the posterior margins; tergite viii with eight long hairs on the posterior margin; tergite ix with a transverse row of four hairs on each side above the posterior margin. Sternites and pleurites with numerous short hairs. The borders of the vulva are closely beset with a row of fine hairs.

MALE.—Resembles the female, except in size. Genitalia with the basal plate short and broad, widest in the middle; parameres elongated and narrow, curved inwards. Near the base of the parameres there is a pair of small plates, each with a well-developed curved hook, and above them another pair of small plates with a similar hook at their posterior extremities.

	Measurements.			
	Female.		Male.	
	Length.	Width.	Length.	Width.
	Mm.	Mm.	Mm.	Mm.
Head	0·44	0·85	0·38	0·76
Prothorax	0·30	0·65	0·28	0·59
Meso- and meta-thorax	0·28	0·82	0·27	0·72
Abdomen	2·04	1·44	1·70	1·05
Total	3·06		2·63	

Holotype: a female.

Described from three females and two males taken off *Platalea alba* Scop. (African spoonbill), at Capetown, Cape Province, in July, 1923.

SUPER-FAMILY *ISCHNOCERA* KELLOGG.

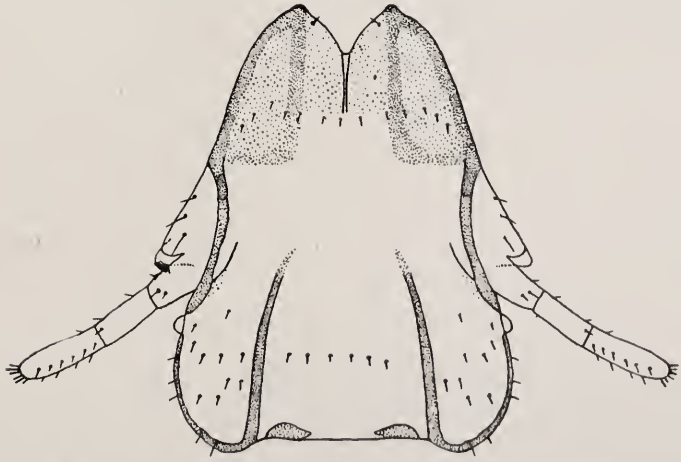
FAMILY TRICHODECTIDAE BURMEISTER.

Genus Tricholipeurus Bedford.

Tricholipeurus lerou xi nov. sp.

(Fig. 7.)

Female.—*Head* (fig. 7) light brown, median area pale, with reddish-brown bands and mandibles. Forehead with about ten hairs on each lateral margin and a transverse row of about thirteen minute



G. A. H. B. del. Fig. 7.

Tricholipeurus lerouxi nov. sp., dorsum of head of female.

pustulated hairs on the dorsum. On the venter there are several similar hairs on each side and a row of four in front of the mandibles; behind, close to the base of each antenna there is a longer hair. Temples with a narrow marginal band and with several minute pustulated hairs on their dorsum and lateral margins. On the dorsum of the hind head there is a transverse row of six minute hairs in the middle. Occipital bands long and narrow, sub-parallel; similar bands present on venter. Signature on gular region triangular. Antennae with the apical segment the longest. Trabeculae triangular.

Thorax same colour as head with narrow, lateral, dark bands.

Pronotum with a minute pustulated hair on each side of the meson and four or five on each side on the posterior margin.

Metanotum with about fourteen similar hairs on the posterior margin. The acetabular bars extend backwards between the mid coxae. Between the hind coxae there are two small pustulated hairs.

Legs with the mid tibiae slightly longer and narrower than the fore and hind tibiae.

Abdomen elongated and narrow, pale in colour, with a brown transverse band and a row of minute pustulated hairs on the dorsum and venter of each segment, the bands on the tergites being wider than those on the sternites. Apical sternites similar to those of *T. trabeculae* Bedford and *T. reduncae* Bedford. Pleurites well developed with several minute pustulated hairs on the posterior margins. Between each of the pleurites i to vi there is a small chitinized spot. Spiracles large, present on segments ii to vii.

	Measurements.	
	Female.	
	Length.	Width.
	Mm.	Mm.
Head	0·44	0·33
Prothorax	0·08	0·25
Metathorax	0·1	0·31
Abdomen	1·05	0·45
TOTAL	1·67	

Described from two females taken off a *Sylviacapra grimmi* (duiker) near the Umfolozi River, Zululand, August, 1929 (Coll. P. L. le Roux), This new species resembles *T. aepycerus* Bedford in the shape of the head, and the apical sternite is similar to those of *T. reduncae* Bedford and *T. trabeculae* Bedford (15th Rep., Dir. Vet. Services, Union of S.A., fig. 19, 1929). The apical sternite of the female of *T. aepycerus* is very distinct (same report, fig. 18).

Genus Lipeurus Nitzsch.

Lipeurus waterstoni nov. sp.

(Fig. 8, *a-d.*)

Female.—*Head* (fig. 8) pale brown in colour with brown to dark brown marginal bands and markings; longer than wide; forehead rounded in front. On the venter there is a row of four hairs situated a short distance behind the anterior margin, and two more, one on each side below the outer pair; between each antenna and mandible there is also a hair. Occipital bands long and narrow. Signature on gular region about twice as long as broad.

Thorax pale with dark brown lateral margins.

Prothorax narrower than the head, lateral margins slightly rounded; on the pronotum at each latero-posterior margin there is a long hair. Sternal plate as in fig. 8c.

Metathorax gradually widening from base to apex; on the dorsum there is a short and minute hair at each latero-posterior angle, and on the posterior margin there are two pairs of long hairs on each side, each pair situated in a small pale area.

Legs with the femora short and broad; tibiae short.

Abdomen with markings and hairs on the dorsum as shown in fig. 8b. Sternites ii to v each with a hair on each side of the meson. Sternite vi with a pair of admedian hairs in front and two pairs behind. Sternite vii with one pair of admedian hairs. Sternite viii with a semi-circular row of short hairs.

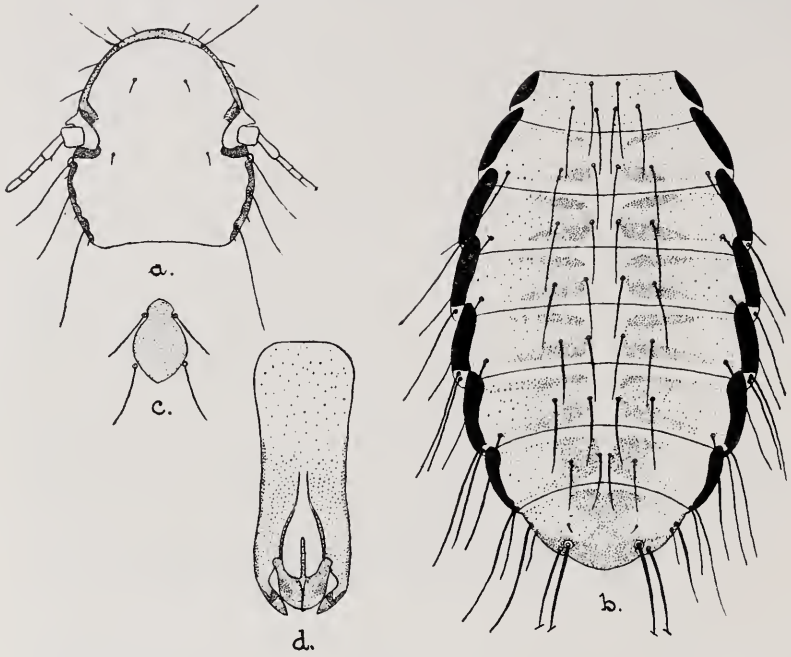
Male.—*Head* and *thorax* as in the female, except that there are five to six hairs on each side on the posterior margin of the metanotum instead of only four.

Abdomen with hairs and bands on tergites i to vii as in the female, except that the latter are more uniform in colour. Tergite viii with transverse bands similar to those on tergite vii, except that they are more curved. Tergite ix pale at the base, brown at the apex, with two hairs on each side and four on the posterior margin. Male genitalia as in fig. 8d.

	Measurements.			
	Female.		Male.	
	Length.	Width.	Length.	Width.
	Mm.	Mm.	Mm.	Mm.
Head	0·57	0·49	0·53	0·43
Prothorax	0·14	0·34	0·14	0·31
Metathorax	0·2	0·52	0·18	0·43
Abdomen	1·32	0·81	0·98	0·64
Total	2·23		1·83	

Holotype: a female.

Described from two females and two males taken off *Scleroptila gariepensis pallidior* Neumann (Ovambo partridge) on the Kunene River, South-West Africa, in March, 1923 (Coll. Dr. R. F. Lawrence).



G. A. H. B. del. FIG. 8.

Lipeurus waterstoni nov. sp., (*a*) head of female; (*b*) dorsum of body of female; (*c*) sternal plate; (*d*) male genitalia.

At first we were inclined to place this species in a new genus owing to the absence of appendages on the antennae of the male, and also to the general structure of the male copulatory apparatus, but after considering the matter over carefully finally decided that it should, at any rate for the present, be retained in the genus *Lipeurus*.

The presence or absence of an appendage on the male antennae is certainly not by itself of any generic importance. For instance, the genus *Rallicola* includes species that possess an appendage on the antennae of the male and also species which have no appendages on the male antennae. The male genitalia of *waterstoni* are certainly very distinct from other known species of *Lipeurus*, but in other respects, namely, the general shape of the head, thorax, and abdomen, chaetotaxy, shape of the sternal plate, and markings on the abdomen of the female, it very closely resembles certain species, especially *L. pternistis* Bedford. The abdominal markings are usually very dimorphic in *Lipeurus*, but in *waterstoni* they are very similar in both sexes.

L. waterstoni is more closely allied to *L. pternistis* than are a number of other species included in this genus.

Degeeriella nigromarginata (Piaget) found on an Asiatic pheasant, *Euplocamus horsfieldi*, is closely related to *waterstoni* and must be included in the same genus.

Genus Coliilipeurus nov.

Species elongated. *Head* similar in both sexes, longer than wide, widest at the temples. Forehead rounded in front, chitinous bands absent, except for a narrow dark band on each side. Eyes well developed. Temporal bands present. Mandibles situated between antennae. Pharyngeal plate small, glands well developed. Antennae 5-jointed, first segment considerably larger in the male than in the female; third segment of male with an appendage.

Prothorax wider than long. *Metanotum* with the lateral margins divergent, posterior margin almost straight with several hairs arranged in pairs, the two admedian pairs being very long.

Abdomen pale, with dark lateral bands and markings, the markings differing in both sexes. Male genitalia with the basal plate short, parameres large and curved, projecting outwards. Species found on Coliidae and Ploceidae.

Genotype: *Esthiopterum colius* Bedford found on *Urocolius indicus transvaalensis* Roberts.

Both *Esthiopterum radiatum* (Neumann) described from specimens taken off an unknown host and *E. nun* (Neumann) found on *Ploceus abyssinicus* should be included in this genus. The latter was described as a variety of the former.

The three species conform to the same type, except that in *colius* (7th and 8th Report, Dir. Vet. Res., Union of S.A., p. 731, pl. 7, f. 1, 2) the mesonotum appears to be distinct from the metanotum owing to a dark transverse band on each side, whereas in *radiatus* they appear from Neumann's figure to be indistinguishable. The transverse abdominal markings shown in the figure of *colius* are ventral, not dorsal.

Genus Naubates nov.

Esthiopterine forms of medium size (about 3.5 to 4 mm.), highly chitinized and brown to dark brown in colour. *Head* shaped as in figs. 9 and 10. Clypeus hyaline. Clypeal signature brown, almost as long as broad, rounded in front, with transverse striae along anterior margin. On the lateral margins of the forehead there is a chitinized band which is interrupted by a narrow clear space; at the interruption the basal portion turns inwards and then backwards, either for a short distance, or continues backwards and inwards, finally joining the band on the opposite side. On the venter of the forehead there is a longitudinal chitinized band on each side. Trabeculae present. Eyes well developed. Temporal bands present. Above the mandibles there is a narrow transverse plate. Pharyngeal sclerite and glands present. On the venter of the hind head there is a longitudinal band on each side extending to the base of the mandibles.

Prothorax wider than long, prosternal plate small, elongated. *Metanotum* with lateral margins slightly divergent, the posterior margin projecting backwards in the middle; on each side near the latero-posterior angle there are four longish hairs arising from a clear space, and in addition to these one or two shorter ones. Sternal plate large.

Abdomen with a dark transverse chitinized band and darker lateral bands on each segment; on the venter the bands are narrower. Spiracles present on the second to seventh segments. Last segment of female bilobed at the apex. Male genitalia with the basal plate of medium length, sides sub-parallel; parameres asymetrical, the one being longer and sometimes broader than the other.

Species found on sea-birds belonging to the families Hydrobatidae and Diomedeidae.

Genotype: *Esthiopterum fuliginosum* (Taschenberg).

In this genus must also be included *N. harrisoni nov. sp.* and *N. pachodroma nov. sp.*

This genus is quite distinct from *Pseudonirmus* Mjöberg, which includes *charcoti* (Neumann) and *gurlti* (Taschenberg), both found on species of *Hydrobatidae*. It is difficult to understand why Harrison sunk *Pseudonirmus* as a synonym of *Esthiopterum*, as the former has priority over the latter.

Naubates harrisoni nov. sp.

(Figs. 12, 14, and 16B.)

Three males and one female taken off *Ardenna gravis* O'Reil (great shearwater) at Capetown, C.P., 1929 (Coll. Dr. R. F. Lawrence). This new species is very closely allied to *N. fuliginosus*, from which it can be distinguished by the shape of the plate on the apical sternites of the female (compare figs. 12 and 11) and the male genitalia (compare figs. 16A and 16B). The longitudinal band on the venter of the forehead is slightly less curved in *harrisoni* than in *fuliginosus*, and the clypeal signature is slightly narrower in the male than in the female, whereas in *fuliginosus* the signature is similar in both sexes.

	Measurements.			
	Female.		Male.	
	Length.	Width.	Length.	Width.
	Mm.	Mm.	Mm.	Mm.
Head	0·98	0·69	1·05	0·67
Prothorax	0·29	0·5	0·29	0·48
Metathorax	0·48	0·71	0·44	0·71
Abdomen	2·39	1·0	2·53	0·87
TOTAL	4·14		4·31	

Holotype: a male.

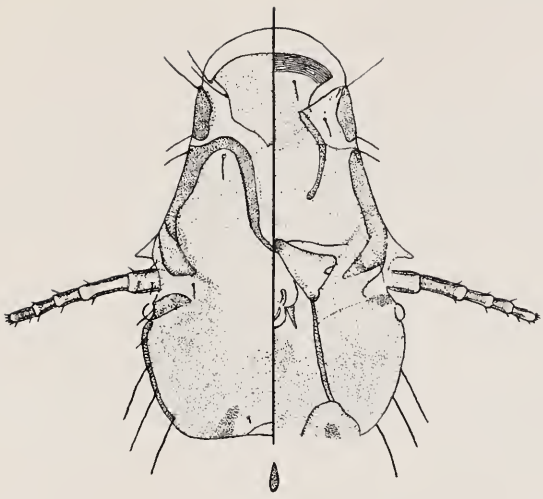


Fig. 9.

Naubates fuliginosus Tasch., dorsum and venter of head and pro-sternal plate of female.

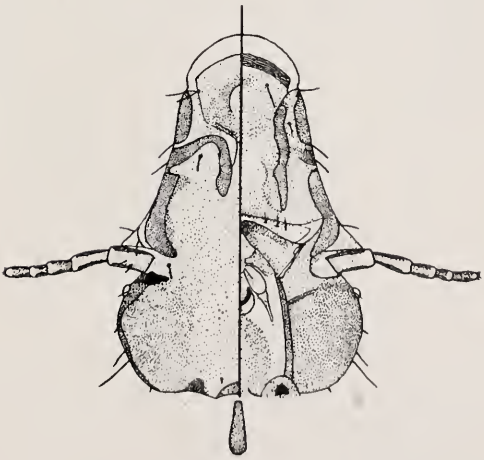


Fig. 10.

Naubates pterodromi nov. sp., dorsum and venter of head and pro-sternal plate of female.

G. A. H. B. del.

Naubates pterodromi nov. sp.

(Figs. 10 and 13.)

Female.—The head is slightly narrower in front of the trabeculae and the temples more rounded than in either *fuliginosus* or *harrisoni* (compare figs. 10 and 9). Other differences to be noted are the shape of the clypeal signature, the chitinized bands on the dorsum and venter of the forehead, and the hairs on the margins of the temples.

Prothorax with the latero-posterior angles acute.

Abdomen dark brown, without hairs on the dorsum, except for one or two near the latero-posterior margins of each segment; tergites ii to vi each with a narrow, indistinct, pale, transverse line extending between the spiracles. On the venter there are two or three hairs on each side of the meson on the posterior margins of the sternal plates, except on the apical plates, which are shown in fig. 13.

	Measurements.	
	Female.	
	Length.	Width.
	Mm.	Mm.
Head	0·89	0·61
Prothorax	0·20	0·42
Metathorax	0·40	0·67
Abdomen	2·10	0·89 (4th segment)
Total	3·59	

Described from three females taken off *Pterodroma macroptera* Gm. (Cape parson), at Capetown, C.P., 1929 (Coll. Dr. R. F. Lawrence).

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FIG. 11.
Naubates fuliginosus Tasch,
apex of apical sternal plate of female.

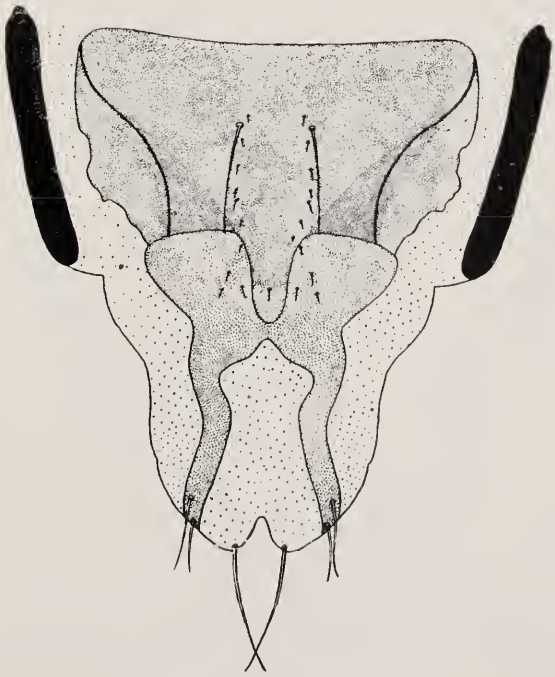

FIG. 12.
Naubates harrisoni nov. sp., apical sternites of female.

G. A. H. B. del.

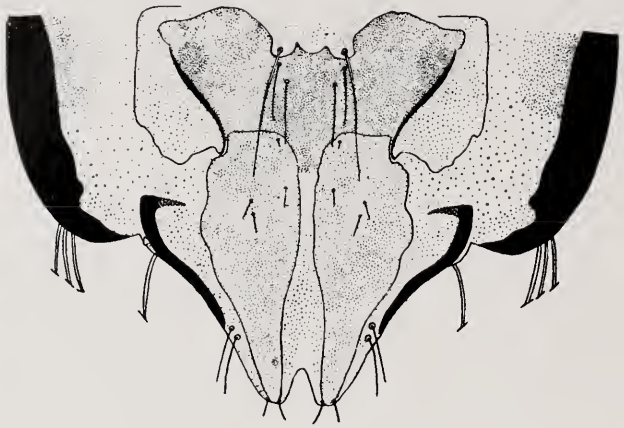

FIG. 13.
Naubates pterodromi nov. sp., apical sternites of female.

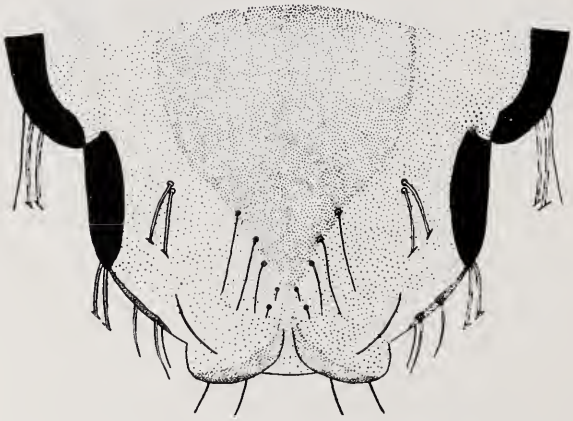

FIG. 14.
Naubates harrisoni nov. sp., apical sternites of male.

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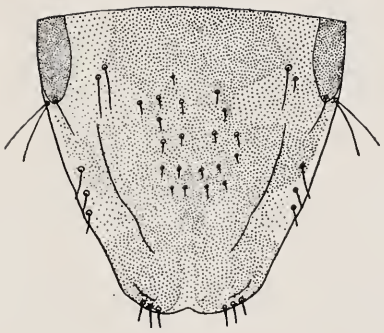

Fig. 15.
Naubates fuliginosus Tasch., apical sternites of male.

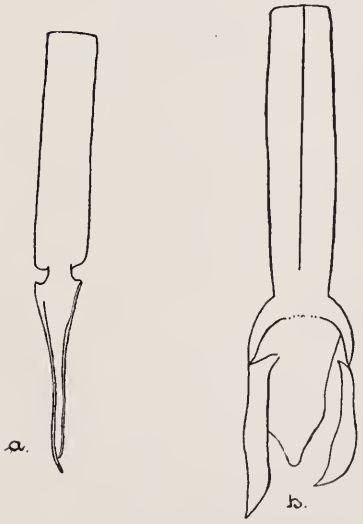

Fig. 16.
Male genitalia of (*a*) *Naubates fuliginosus* Tasch., (*b*) *N. harrisoni* nov. sp.
G. A. H. B. del.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

Studies on the Bionomics of the Free-Living Stages of Trichostrongylus Spp. and other Parasitic Nematodes.

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Veterinary Research Officer, Onderstepoort.

In a previous paper, Mönnig (1926), the development of *Trichostrongylus instabilis* and *T. rugatus* was described with reference to the free living stages as well as the parasitic stages in sheep. The following pages contain a brief account of the results of studies on the bionomics of the free-living stages made during the last seven years, with comparative studies on the larvae of other parasitic nematodes. The results of other workers on the bionomics of related nematode larvae are discussed for comparison of the habits of these larvae and in discussions of technique employed.

PURE INFECTIONS.

In studies of this nature it is frequently necessary to work with the droppings of animals infected with only one species of nematode. Only in this way can one obtain large numbers of eggs or larvae of a single species, which is essential where a study of the effects of certain conditions on the larvae is to be pushed to finality—it is evident that the resistance of e.g. 0.1 per cent. of larvae against a certain factor will only be seen when a large number are used and, since pastures may be infested with many millions of larvae, even such small percentages are significant.

Veglia (1928) and others, have described methods of raising lambs free of worm infection. Such methods are excellent and often indispensable, but impose many restrictions. It was found at Onderstepoort that, where sheep are kept in small gravelled paddocks, lambs born in the beginning of the dry winter are almost always free of infection until the rains start in spring. This fact was frequently made use of. Otherwise, since the sheep at the Institute rarely have other worms than *Haemonchus*, *Oesophagostomum*, *Trichostrongylus* and *Strongyloides*, infected lambs were treated to expel the worms and were then kept in small stables with sawdust on the floors, which were cleaned without the use of water twice a week. *Strongyloides* infections were usually disregarded, since they did not cause any trouble in the tests and because even under the most careful attention to the sheep these worms would appear and disappear in them; occasionally infections with the other species also suddenly appeared in these sheep but on the whole they were not very troublesome.

If a pure culture of one species is not available to infect a clean lamb, larvae of the species required can be picked up from a mixture of larvae in water by means of a fine pipette, working under a binocular microscope.

As will be seen later, it was found that eggs of *Trichostrongylus* spp. containing complete embryos are very resistant to desiccation, while this is not the case with the other parasites mentioned above. In order to obtain pure cultures of infective *Trichostrongylus* larvae, sheep faeces containing eggs of different species were incubated for 18 hours, then dried and moistened again, after which only *Trichostrongylus* larvae developed in them.

In the case of *Oes. col.*[1] a lamb was successfully infected in the following way: For 48 hours the lamb was given dry food and no water. An older sheep infected with *Oes. col.* was then killed and about 500 of these worms were collected in warm physiological saline. Immediately afterwards the worms were administered in a small quantity of saline to the lamb through a funnel and rubber tubing pushed as far as possible into the colon through the anus, while the lamb was held up by the hind-legs. A small quantity of saline was then given to wash down any worms sticking in the tube. When the latter was withdrawn it was found that all the worms had been passed into the lamb. During the next 24 hours the lamb passed about 200 of these worms but the rest remained and the lamb immediately started passing their eggs in its droppings and continued to do so for several weeks, after which the infection disappeared.

A discussion of methods of making faeces cultures will be found under the heading " Geotropism and Migration from Cultures."

Since the parasites concerned are most frequent and do most damage to sheep in those parts of the Union where the rainfall occurs chiefly in summer, while the winter is practically dry, the experiments have been planned accordingly and were carried out under such conditions.

EGGS AND PRE-INFECTIVE LARVAE.

The periods of development of the free stages of *Trich. instabilis* and *T. rugatus* under optimal conditions have been given in the paper mentioned above and are briefly as follows: Eggs hatch 19 hours after having been passed by the sheep, the first ecdysis takes place 25 hours later, the second ecdysis 19 hours after the first, the infective stage being reached under optimal conditions in 63 hours.

It is well known that warm, moist conditions are ideal for development, while cold and dryness retard the process or cause death of the eggs and larvae. In order to determine the possibilities for development of *Trich.* spp.[1] under the cold, dry winter conditions the following experiments were made.

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[1] For the sake of brevity *Oesophagostomum columbianum* will be referred to as *Oes. col.*, *Haemonchus contortus* as *H. cont.*, *Trichostrongylus* as *Trich.*, and *Strongyloides papillosus* as *Str. pap.*

[1] The species of *Trichostrongylus* used in these investigations was chiefly *T. instabilis;* sometimes *T. rugatus*, *T. falculatus*, or *T. axei* were present.

Field Tests.

EXPERIMENT 1.

Faeces of lambs with pure infections of *Trich.* were used. Fresh droppings in the form of pellets were placed out in small heaps as follows on 14th May, 1924:—

1. Under tree, in grass, left open and dry.
2. „ „ left open and kept moist.
3. „ „ under glass bell-jar, left dry.
4. „ „ under glass bell-jar, kept moist.
5. „ „ under dark bell-jar, left dry.
6. „ „ under dark bell-jar, kept moist.

1*a* to 6*a* respectively, like the above but not under a tree.

Control cultures were kept in jars in the laboratory at 25° C. Records were made of day and night temperatures and weather conditions, and samples of the faeces were regularly taken for closer examination. It is not necessary to give all the details here; the following are the most important observations. On the whole there was little wind; a heavy dew fell the second and third nights and a very light rain came unexpectedly the sixth night, after which the weather was dry day and night. The maximum (day) temperatures varied from 17.2° C. to 25.5° C. and the minimum (night) temperatures from 0.5° C. to 6.6° C. The development proceeded as follows:

1 and 1*a* due to the dew and rain the faeces obtained moisture at intervals and on the seventh day some of the eggs in the centres of the heaps hatched; then evaporation proceeded rapidly and on the eighth day all eggs and larvae had become dry and did not develop further.

2 and 2*a*.—Development proceeded slowly and a good number of larvae reached the infective stage on the ninth day; there were more in 2 than in 2*a*.

3 and 3*a*.—The moisture of the faeces evaporated and condensed on the sides of the bell-jars and then gradually disappeared. Some larvae hatched but everything dried up on the 5th day and development stopped.

4, 4*a*, 6 and 6*a*.—Results as in 2.

5 and 5*a*.—On the whole like 3 and 3*a*, but a few larvae reached the second stage.

In control cultures larvae reached the infective stage on the fourth day.

It is seen that, due to the low temperature and consequent slower development, drying overtakes the exposed larvae and kills them before they reach the infective stage. With a nightly fall of dew a part of the faeces may be kept moist but the infective stage could only be reached if the moisture should be kept up sufficiently long and the faeces lie thickly enough, which is not usually the case where sheep defecate on a pasture. At the time of the experiment the day temperature was still fairly high, causing more rapid evaporation but also more rapid development than would be the case later in the winter.

A similar experiment was made in July under more severe winter conditions and the effects of cold and dryness were more accentuated. A somewhat similar experiment, also made in July, is here recorded.

EXPERIMENT 2.

Fresh droppings of sheep with pure *Trich.* infection were placed in dry grass about 10 cm. high, next to a small cypress tree which shaded them from 9.30 a.m. till 2.30 p.m. Specimen A consisted of small heaps of rather moist pellets, while specimen B consisted of soft, moist faeces in the form of balls 3-4 cm. in diameter. There was little wind and only a light frost fell each night. The maximum (day) temperatures were 20° C. to 23° C., the minimum (night) temperatures, 4° C. to 0° C. In the case of A the pellets dried up rapidly and were completely dry to the centres of the heaps on the third day; the maximum stage of development reached was the tadpole embryo. In specimen B development and drying in the outer layers proceeded in a similar way as in A, while in the inner portions at first no development took place on account of absence of air. As the outer layers dried out and the air gained access further inwards the eggs here started to develop and a few even hatched before the whole mass finally dried out.

Even in initially moist balls of faeces therefore drying kills the larvae long before the infective stage is reached. It can thus be concluded that *Trich.* larvae can only exceptionally reach the infective stage under winter conditions. Such exceptional conditions would be found at the edges of pools or streams of water, or when heavy dew falls constantly or with continual rains which are, however, very rare in the winter in this country.

The following experiments were made to test the individual factors singly.

Effect of Dryness.

In the above experiments, when a specimen had dried out, it was taken that the pre-infective stages were dead, since development stopped and since this was accepted to be the case also for *H. cont.* and *Oes. col.* according to the work of Veglia (1916 and 1923) and others. It was, however, necessary to test this point more accurately. As the results of these experiments led to more and more tests, the list of these tests became quite a long one and only the most important points can be recorded here. The method employed was as follows: Normal, fresh droppings were taken from sheep with known and fairly heavy infections and these droppings were crushed and incubated in the ordinary way, while the development of the eggs or larvae was watched by examination of samples taken at frequent intervals. A sample of faeces evacuated by a sheep in the course of several hours naturally contains eggs in different stages of development and the variation is sometimes considerable. When the majority of eggs or larvae had reached the desired stage, the faeces were spread thinly on a tray and placed in a draught (not in the sunlight) to dry quickly.

A small sample was not dried but further incubated as a control to see if development to the infective stage would proceed normally.

The dried faeces were placed in large jars which were covered so as not to exclude access of air, and the jars were kept in a dark cupboard in the laboratory.

At regular intervals about two tablespoonfuls of faeces were moistened, placed in a suitable tube and incubated. After seven days of incubation the contents of the tube would be transferred into a unit of a Baermann separating apparatus and the larvae removed by this method identified and counted after 24 hours. The apparatus had been previously tested for its efficacy in removing larvae of the worms concerned from soil and from faecal cultures as will be described later.

The results obtained showed the following: Eggs of *Trich.* in the morula, tadpole and further stages, before complete embryonic development had been reached, never recovered after the faeces had once been dried to a light, dusty condition. Faeces containing *Trich.* eggs with complete embryos and younger or older pre-infective stages, when moistened after drying, contained infective *Trich.* larvae on the seventh day.

In order to determine which stage exactly is the resistant one, first and second stage larvae were separated from cultures by means of the Baermann apparatus—even for such larvae the apparatus is suitable— and were then dried on slides. These larvae never revived after having been dry for even a few hours. It must therefore be concluded that it is the egg containing a complete embryo which is resistant.

The degree of resistance is exceedingly great and is even much greater than that of the infective sheathed larvae. In the first number of tests the material was not sufficient to reach the end point of the resistance. In some cases an end point was apparently reached but even in the specimen (No. 20) which contained the largest number of eggs and in which the longest period of resistance was determined, the end point was not reached because the material was used up when some larvae still could develop from it. This is a good example of the necessity of working with large amounts of material, which has been discussed before.

In various specimens larvae were found to develop after the faeces had been dry for 199, 299 and 389 days. In specimen No. 20 about 150 larvae were found in the sample taken after the faeces had been dry 1 day, 30 after 9 months, 7 after 13 months, 2 after 14 months and 1 after 15 months.

It can, therefore, be concluded that eggs with complete embryos of the *Trich.* species used can resist dryness for 15 months or longer, but that younger or older pre-infective stages do not resist dryness. Contrary to these results Zawadowsky (1929a) states that *Trichostrongylus* eggs do not resist desiccation.

Similar tests were also made on *H. cont.* and *Oes. col.* but none of their pre-infective stages were found to recover after having once been dry. Veglia (1916) performed somewhat similar tests on *H. cont.*, from which it would appear as if eggs with complete embryos could resist drying for a few days only. Ransom (1906) states that eggs and pre-infective stages of *H. cont.* are speedily killed by drying.

From a study of the relative literature it seems as if this phenomenon has been noted in only two other instances, viz., by

Looss (1911, p. 396) in the case of *Strongylus* spp. of the horse, of which completely embryonated eggs resisted desiccation for 6 but not for 9 months, and by Theiler and Robertson (1915) in the case of *Trichostrongylus douglassii*, in which case faeces, containing eggs and first stage larvae, kept dry for 2 years, gave rise to mature larvae when moistened, or resisted desiccation over calcium chloride for 30 but not for 36 months. It should be interesting to carry out similar tests in the case of other nematodes with a similar life-history where no definite observations have been made, and it is undoubtedly a point of great practical importance. It would thus be possible for sheep to infect a pasture with *Trichostrongylus* even in the cold, dry winter, if the eggs can just reach the complete embryo stage, which may easily happen, although here the possible harmful effects of direct sunlight and cold on the eggs must also be considered.

Effect of Temperature.

There is an optimal temperature for development for practically every living organism; lower or higher temperatures cause slower or more rapid development and frequently deterioration or death. Among the *Strongylid* nematodes it has generally been found that temperatures near freezing point arrest development and that degeneration may take place (Schwartz, 1925, and Sprehn, 1925, on *Bunostomum phlebotomum*, De Blieck and Baudet, 1926 on strongyles of the horse, etc.), or that freezing may cause death of the eggs or larvae in a short time (Boulenger, 1915, on *Nematodirus filicollis*, Ransom, 1906, and Veglia, 1916, on *H. cont.*, etc.). Temperatures not far below the optimum usually only cause slower development. Again, temperatures that are too high (e.g. 37° C.) may cause rapid initial development and then death of the embryo or young larval stages almost invariably follows as e.g. in *Trich. douglassii* (see Theiler and Robertson, 1915), *Nematodirus filicollis* (see Boulenger, 1915), *Dictyocaulus filaria* and *D. vivipara* (see Daubney, 1920), etc. In the case of the *Trich.* spp, observed the conditions are very similar to the above. All the pre-infective stages are fairly rapidly killed by actual freezing. But it seems that, where there is a certain amount of protection, these stages may resist cold for a fair period period of time (see Exp. 2).

EXPERIMENT 3.

Crushed fresh faeces of infected sheep in closed glass jars, as used in the laboratory for cultures, were placed on several occasions during the winter in the shade of a tree or bush or on the south (cold) side of a building so that they got no direct sunlight. The night temperatures were very frequently below freezing point, yet development proceeded slowly up to a time when the larvae died, not having reached the infective stage. In one such case the night temperature was usually below freezing point and the day temperature not above 15° C.; the jar stood in the open at a very cold spot, in permanent shade. The eggs hatched after 10 to 18 days and a few larvae completed the first ecdysis some 15 days later but then the infection rapidly died off due to the low temperature, moulds or altered conditions in the medium or a combination of these factors. From experiments 1 and 2 recorded above, it would also seem as if the cold alone, under the conditions of these observations would not be sufficient to kill the pre-infective stages sheltered in faeces.

Temperatures above the optimum, which is about 26° C., cause more rapid development, but degeneration follows in the case of the higher temperatures. At 37° C. the embryonic development was completed very rapidly, but rarely an egg hatched, or the larvae died soon after. Altered conditions in the medium may play an important part here, but eggs in a thin layer of pure water also degenerated.

Viability of Eggs under Water.

It is a well known fact that the eggs of Strongyles and other nematodes require oxygen for their development and that they do not develop in water deeper than about 3 mm. This is also the case with *Trich.*, *H. cont.*, and *Oes. col.* Theiler and Robertson (1915) state that eggs of *Trichostrongylus douglasii* remain viable under water for two months. Veglia (1916) found that " in liquid medium kept immobile and deeper than .5 cm. a rather small percentage of the eggs (of *H. cont.*) hatch after four days, but the larvae made very little progress, and within 12 to 15 days death occurs, presumably owing to lack of air." The eggs that had not hatched were dead.

EXPERIMENT 4.

In order to find how long eggs may remain viable under water, faeces of sheep with pure infections of *Trich.*, *H. cont.* and *Oes. col.* respectively were broken up in water, strained through a set of sieves and the fine faecal particles and eggs which passed through were placed in glass dishes under 3 cm. of water. The dishes were covered so as to allow access of air and were kept on the laboratory table in mild diffused light. At regular times samples of the sediment were removed, mixed with sterile sheep faeces and incubated; a control sample was incubated at the beginning. At other times samples were spread out in petri dishes and it was only noted whether the eggs hatched.

In a large number of cases the eggs did not remain viable for more than a few days. Also in this case one should work with large numbers of eggs. After repeated tests the longest periods determined that the eggs remained viable were 13 days for *Oes. col*, and 45 days for *H. cont.* and for *Trich.* the contents of the eggs could be seen to decompose and numerous bacteria and infusoria developed in the specimens. No eggs hatched or even developed under water.

MATURE OR INFECTIVE LARVAE.

As is the case with other Strongyles, the infective larvae of *Trich.* differ very much in their habits from and are more resistant to various factors than the pre-infective stages. In general they show the various tropisms that are shown by the larvae of *H. cont.*, so ably described by Veglia (1916).

Phototropism and High Temperatures.

Generally the infective larvae of Strongyles are attracted by a mild diffused light or are at least activated by it, but they are repelled by strong light. This also is the case with *Trich.*, *H. cont.* and *Oes. col.* Some larvae are killed by sunlight, e.g. *Trich. douglassii* (see Theiler and Robertson, 1915), and *Heligmosumum muris* (see Yokogawa,

1922), while others are not so seriously affected, e.g. *Ancylostoma* and *Necator* larvae according to Looss (1911) and the larvae of *Bunostomum trigonocephalum* which, according to Cameron (1923) suffer no harm from lying in front of a window, although this probably does not imply direct sunlight. Also the larvae of *Ostertagia circumcincta* are not killed by sunlight according to Morgan (1928). In judging the degree of phototropism of larvae from their behaviour on the walls of culture jars placed near a source of light, one should take into account the presence of moisture on the walls of the jar. Frequently the moisture condenses on one side of the jar and naturally the larvae can migrate up this side only. Under such conditions *Trich.* larvae could frequently be found either on the wall facing the light or on the opposite wall, whichever was the moist one.

EXPERIMENT 5.

A large number of *Trich.* larvae were placed in tapwater in a glass tube 50 cm. long by 0.7 cm. thick, closed at both ends. One half of the tube was pushed into a cardboard thermometer case and the opening around the tube plugged with black wool. The tube was placed level on the table in strong diffused light and in another case in direct sunlight. As is usually the case with larvae in water, some became inactive and did not move. The others near the closed portion soon migrated into it. On pulling out a small portion of the covered tube a mass of larvae was seen to have gathered there from both halves. Further exposure caused this mass to move into the beginning of the closed portion again, where the light was just strong enough for them.

Direct sunlight does not kill the larvae of *Trich.* very quickly but it is undoubtedy harmful. Under natural conditions the larvae will, however, usually be able to seek protection and hence the effect of direct sunlight was not extensively studied.

The effects of high temperatures were studied in field experiments —see under " Longevity of Larvae "—and by placing cultures with infective larvae in an incubator. It is difficult in any of these tests to exclude other factors while the larvae are not kept under unnatural conditions.

EXPERIMENT 6.

Placed in an incubator, in diffused light at 37° C., the larvae that had just reached the infective stage and migrated up the walls of the jar, migrated downwards and did not come up again. They gradually died off and in 12 days all were dead. After a few days at 37° C. the larvae that were still alive did not migrate upwards when the jar was placed on the laboratory table. Naturally one has to think of decomposition in the medium affecting the larvae, but it is strange that they did not leave this medium and that the migratory instinct should have been so affected by the temperature. In the next section a similar reaction to cold will be described.

Low Temperatures.

Ransom (1906) states that *H. cont.* infective larvae in faeces resisted 85 days' exposure outside in the winter, being frozen and thawed out 32 times and frozen for over 48 hours on three occasions.

Veglia (1916) reports that *H. cont.* infective larvae on blotting paper resisted freezing for 6 months, when 5-6 per cent. recovered. A number of other species of larvae have also been found to resist freezing but the tests were usually not carried out over such long periods (Ortlepp, 1925, on *Triodontophorus tenuicollis*, Schwartz, 1925, on *Bunostomum phlebotomum*, Theiler and Robertson, 1915, on *Trich. douglassii*, De Blieck and Baudet, 1926, on Strongyles of the horse, etc.). Although one could probably take it for granted, it was rarely determined whether the larvae could still infect a host after such exposure. In other cases freezing or low temperatures kill the larvae as in the case of *Bunostomum trigonocephalum* according to Cameron (1923).

EXPERIMENT 7.

Infective larvae of *Trich.* placed in water in a watch glass on a block of ice overnight did not seem to be affected by the low temperature. In fact, in one case they were moving about in the water, which had not frozen, more lively than larvae would usually do in water at room temperature. In other cases the low temperature caused cessation of the movements.

EXPERIMENT 8.

A large number of larvae were placed in water in a small bottle and the latter was corked and immersed in a brine freezing mixture used for making ice. The temperature must have been well below 0° C. After 10 days the bottle was removed and the contents were frozen hard. After thawing was completed the larvae were found to swim about lively and they were drenched to a clean lamb, which later proved to have become heavily infested.

EXPERIMENT 9.

A culture jar, with larvae that had just reached the infective stage and had migrated up the sides of the jar, was placed in a refrigerator running at 0° C. The larvae descended into the faeces. The jar was removed from the refrigerator after 14 days and, although the larvae were still alive, they did not migrate upwards again during the next two days as long as the observation was continued, although the conditions for migration were quite suitable.

Daubney (1920) states that the larvae of *Dictyocaulus filaria* and *D. arnfieldi* may lose the power of migration when subjected to low temperatures.

Resistance to Dryness.

Numerous observations have been made on the resistance of infective larvae of various species to desiccation. The results of some authors are directly opposed to those of others; e.g. De Blieck and Baudet (1926) and Baudet (1925) state that infective larvae of horse Strongyles and Trichonemas do not resist desiccation, while others find them to be rather resistant (Looss, 1911 and Cameron, 1923). The results of *Dictyocaulus filaria* larvae are also conflicting, although it is probable that they are quickly killed by desiccation.

Most of the skin penetrating forms do not resist desiccation (e.g. *Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis*), while non-skin-penetrators are usually resistant. But this cannot be accepted as a general rule, as Schwartz (1925) has pointed out, since species like *Dictyocaulus arnfieldi, Bunostomum phlebotomum* and *B. trigonocephalum* do not resist desiccation. Beller (1928) claims that the latter species is a skin penetrator but the question cannot be accepted as settled.

Concerning the larvae of *Trich.* spp. and *H. cont.* there are also conflicting statements in the literature. Theiler and Robertson (1915) found that *Trich. douglassii* larvae resisted desiccation for 9 months. Zawadowsky (1929) states that a small percentage of larvae of the *Trich.* of sheep revive after 2 months of dryness. Ransom (1906) found that *H. cont.* larvae dried in faeces for 35 days could recover. Veglia (1916) placed *H. cont.* larvae in faeces under a tree where they dried out; after 3 months 50 per cent. revived. Concerning *Ocs. col.* Veglia (1923) states that this species closely resembles *H. cont.* in its tropisms and resistance. Daubney (1928) states, concerning *H. cont.* larvae, that they do not posess any very great powers of resistance against desiccation, although in this respect they are not quite so defenceless as the hookworm larvae; also that *Trich.* larvae are similar to *H. cont.* in their resistance to desiccation. Although Daubney's experiments were made in Kenya, it is strange that his results should differ so greatly from those of other workers.

The conditions of exposure are important, e.g. the presence or absence of débris around the larvae, as has frequently been pointed out by different authors. Moreover, the relative humidity of the atmosphere and therefore also the temperature and the time of the year are very important, as was brought out clearly in the experiments of the writer. All these are, of course, factors which determine the *actual degree of "dryness"* of the larvae and this degree of dryness is the really important factor on which the result depends. It would, therefore, be desirable to state in all experiments of this nature what the actual conditions were and, for very exact work, it would be necessary to work in an atmosphere of known or controlled humidity and temperature.

A large number of tests on the resistance of *Trich., H. cont.* and *Oes. col.* larvae have been made by the writer during the last years. All these tests were made in the same way, viz. by removing larvae free of débris from the walls of culture jars, placing these into drops of water on slides and allowing the water to dry away. The slides were then kept in slide-covers, not completely closed, standing on the laboratory table. Whenever any work was done during which possibly poisonous gases or fumes escaped into the air, the dry larvae were first removed to another room. There were about 10,000 larvae on each slide. At fixed times a specimen would be moistened, placed under a glass cover and repeatedly examined until no more larvae recovered.

The longer the larvae had been dry, the longer did they require to revive. Frequently the larvae cast their sheaths when they revived; this happened in all three species (*Trich., H. cont.* and *Oes. col.*)

EXPERIMENT 10.

Specimens of Trich. larvae were dried on 5th September, 1927 (Spring, with rising temperature and dry atmosphere, the rains started late). After a week only about 5 per cent. still recovered on moistening. No more larvae revived after the 50th day, when 0.5 per cent. recovered.

EXPERIMENT 11.

A parallel test to Experiment 10, keeping the dried larvae in a refrigerator running at 0° C., which means that very little evaporation would take place, but that the larvae also would get very little moisture from the atmosphere. After 7 days 40 per cent. revived, after 30 days 10 per cent. The specimens then were accidentally moistened.

EXPERIMENT 12.

On the 1st March, 1929 (Autumn, temperature falling, atmosphere moist) *Trich.* larvae were dried. Up to the end of March the atmosphere was moist, light rains falling frequently. From April onwards the temperature was fairly cool and the atmosphere dry. A light rain fell in the beginning of June. After 10 days 85 per cent. recovered, after 30 days 60 per cent., after 64 days 40 per cent., after 100 days (just after the rain in June), 45 per cent., after 115 days on the 26th June, 1929, still 45 per cent. The material was then unfortunately finished.

EXPERIMENT 13.

This can be considered to be a continuation of Experiment 12. The larvae were dried on the 4th April, 1929. It was cold and dry during July and August. In September the temperature rose, but it rained on the 10th September, 1929 and after that the atmosphere was fairly moist. After 83 days (26th June, 1929), 45 per cent. revived. After 171 days (16th September, 1929), 5 per cent. revived, after 7 months 0.5 per cent., and finally after $8\frac{1}{2}$ months 0.1 per cent. still recovered. The resistance dropped rapidly when the temperature increased and while it was still dry.

Similar experiments were made with *H. cont.* and *Oes. col.* but not at quite such favourable times of the year as Experiment 13 was done. Larvae dried in September, 1927, did not live longer than 28 days in the case of *Oes. col.* and 65 days in the case of *H. cont.* Larvae dried in the beginning of winter lived much longer and the longest period determined for *H. cont.* was $4\frac{1}{2}$ months and for *Oes. col.* 3 months.

From comparative tests during warm, dry times, when the larvae did not live very long, it appeared that *H. cont.* and *Trich.* were about equally resistant while *Oes. col.* was less resistant.

From the above results it is evident that a cool, moist atmosphere is most favourable while a warm dry, atmosphere is very unfavourable. This should be quite obvious. Warm air can absorb much more water vapour than cold air and the actual degree of dryness of larvae in warm dry air, will be much greater than in cold dry air. Moreover, when the atmosphere is saturated or nearly saturated with

water vapour, it seems as if the dry larvae can absorb moisture from the air, as was seen in Experiment 12 when more larvae revived after the rain in June than had been the case just before the rain.

That the higher temperature itself probably has no deleterious effect on the dry larvae is seen from Experiment 13. When the temperature rose in spring the percentage of larvae recovering decreased rapidly until the rains started and then the rate of decrease lessened considerably. In this connection it may be recalled that, according to Boulenger (1915), the mature larvae of *Nematodirus filicollis* resisted 10 hours dryness at 60° C. and 2 hours at 70° C.

After recovery from a prolonged period of dryness, the larvae show considerable changes in the intestinal cells. The number of reserve food granules have decreased and gas bubbles have accumulated in quantities, proportional to the length of the period of dryness. Here again, although one could take it for granted, it has rarely been proved that such larvae are still infective, although this is a very important point. In tests made with *Trich.*, *H. cont.* and *Oes. col.*, it was found that larvae recovering after 3 weeks of dryness were able to infect lambs.

Viability under Water.

Since pools of water in the pasture, from which sheep often have to drink, may contain large numbers of larvae which have been concentrated here from the surrounding higher ground through the action of rains, it is important to know how long the larvae may live under these conditions. Most of the tests of this nature hitherto made on larvae of different species have been carried out with larvae in clean water. Some of the results are tabulated here for comparison.

Species.	*Viability in Water.*	*Author.*
Trich. douglassii	13 months	Theiler and Robertson (1915).
Nematodirus filicollis	11 months	Boulenger (1915).
Strongyles of horse	4 months	De Blieck and Baudet (1926).
Ostertagia circumcincta	3 months	Morgan (1928).
Bunostomum trigonocephalum	at least several months	Cameron (1923).
Haemonchus contortus	5½ months	Veglia (1916).
Strongyloides stercoralis	5 days	Fülleborn (1914).

EXPERIMENT 14.

About 100,000 infective larvae of Trich. were placed in tap-water 10 cm. deep in a glass dish on the 4th February, 1929. The dish was kept on the laboratory table and was protected by a glass cover which allowed access of air at the bottom. Fresh water was added monthly to restore the level which had dropped owing to evaporation. Since the water was fairly hard, a fair amount of lime salts collected in the dish in the course of time. The number of larvae decreased noticeably from the second month. The last live larvae were seen after 7 months. At the bottom of the vessel bacteria developed and gradually a granular, gelatinous substance was formed. The larvae that died decomposed and disappeared; dead larvae were never seen, only decreasing numbers of live ones.

Veglia (1916) mentions that from the first month onwards many *H. cont.* larvae cast their sheaths. In the case of the *Trich.* larvae unsheathed larvae were not seen but it may possibly be that they cast their sheaths and then became susceptible to bacterial attack which destroyed them.

EXPERIMENT 15.

Two aluminium vessels of 40 cm. diameter and 17 cm. deep were filled with 5 cm. mud and 8 cm. water. One (A) was placed on the verandah so that the sun could shine into it for a few hours each day, while the other (B) was placed in the laboratory in a fairly dark corner. To each vessel many thousands of infective larvae of *Trich.*, *H. cont.*, *Oes. col.* and *Str. pap.* were added on the 21st May, 1929. At fixed times specimens were taken, by means of a 20 c.c. pipette from the surface of the mud, and examined under a binocular microscope, individual larvae being removed for more detailed examination when necessary.

During the first month the numbers of larvae did apparently not decrease except *Str. pap.* which died very soon. On the 26th June, 1929 there were noticeably fewer larvae in A while B had not changed. In A there had developed numerous Crustacea and Spirogyra began to grow but was frequently removed so as not to obstruct the action of the sunlight. On the 10th July, 1929 a decrease in the number of larvae could also be noticed in B. On the 9th August, 1929 only a small number of larvae of all three species could be found in B; in A no larvae were seen under the binocular but a sample of mud placed in the Baermann apparatus yielded a few larvae of all three species. On the 9th September, 1929 B was still infected but no more larvae were found in A. The last larva recovered from B by the use of the Baermann apparatus was a *Trich.* larva on the 9th October, 1929, i.e. $3\frac{1}{2}$ months from the beginning.

The larvae, therefore, do not live as long in water containing mud and small invertebrates as they do in tapwater and they die off sooner in water exposed to sunlight than in water in the shade. Whether the sunlight is directly or indirectly harmful is of no great practical consequence. The temperature of the water did not rise very much.

Geotropism and Migration from Cultures.

This subject also has been much studied with various species of larvae. A review of the literature, however, creates the impression that there is a great lack of uniformity in the technique of such tests and that this is reflected in the results. Nematode larvae that do migrate from cultures do so only under certain conditions of temperature, light, moisture and consistency of the culture medium. It is in the latter point that the experimental conditions of different workers vary and which is the cause of conflicting results. No better example can be quoted (without the intention of any criticism) than that of Cameron, who in 1923 reported that the larvae of *Bunostomum trigonocephalum* do not migrate from faeces cultures and in 1927 found that they did migrate, the difference in the two cases being the consistency of the culture medium.

Goodey (1924) states that the infective larvae of *Oes. dentatum* of the pig are positively geotropic and do not migrate from cultures. The writer specially tested this point by mixing infected pig faeces with sterilized sheep faeces into a semi-solid medium and found that the *Oes. dentatum* larvae migrated very well up the sides of the jar. The larvae were carefully identified and, needless to say, no larvae developed in control cultures of the sterilized sheep faeces alone. Goodey's statement may sound feasible, because pigs could perhaps become more easily infected by larvae remaining on or in the soil than by larvae migrating on to the stems and leaves of plants, but then this species of *Oesophagostomum* would differ considerably from *Oes. col.*

Also Baudet (1925) states that *Trichonema* larvae do not migrate from cultures. In the writer's experience they have always migrated when the conditions were suitable.

It should not perhaps be necessary to discuss this matter any longer, because it has frequently been pointed out by Looss and others, that nematode larvae cannot migrate upwards out of water or a very wet medium, the surface tension preventing the larvae to leave the water, as can easily be seen by watching the actions of larvae in a drop of water under the microscope. However, it is very desirable to reach greater uniformity and more consistent results. For this purpose, cultures to be used in testing geotropism and migratory activity, should be made in a more uniform way. Veglia, in his publications, has described the methods used at the Onderstepoort Laboratory and has also pointed out the advantages of using sterilized sheep faeces to replace charcoal in cultures with sheep faeces. The writer has used sterilized sheep faeces to mix with the faeces of sheep, cattle, pigs and the dog with great success. If the faeces are solid and dry enough no addition is necessary. If not, then the required amount of sterilized sheep faeces are added (fairly dry) to proudce a medium resembling in consistence and moisture content that of normal, fresh sheep pellets broken down into a loose mass. This mass is placed into 2-pint size or larger fruit jars, which are not more than half filled, and the inside wall of the jar is wiped down clean. The faeces are then slightly heaped up against the wall of the jar so as to leave a pit in the centre; experience has shown that more larvae migrate up the wall when this is done and an explanation may be found in the discovery of Augustine (1923), that human hookworm larvae do not migrate much in a horizontal but rather in a vertical plane, which may hold for other species of larvae as well. The jars are then firmly closed and kept at a temperature of 26° C. in a dark room. To see migration, or to obtain larvae, the jar is placed in a mild diffused light at room temperature when the larvae have reached the infective stage and they soon appear and can be removed free of débris. Larvae that do not migrate can usually be separated by the Baermann apparatus. Under such conditions the infective larvae of *Trich.*, *H. cont.*, *Str. pap.* and *Oes. col.* migrate upwards in a diffused light but not in a strong light. They all behave in the same way as Veglia (1926) has described in detail in the case of *H. cont.* The same author mentions that *H. cont.* larvae migrate for about 20 to 30 days under artificial conditions and that they then

remain in the culture medium. The same happens in the case of *Trich.*, although larvae of *Trich.*, *H. cont.* and *Oes. col.* are sometimes found on the walls of the jars in cultures 2-3 months old.

Longevity in Soil.

In order to study the longevity of larvae in soil, a technique for separating the larvae from soil must be employed and the larvae must be identified so as to discriminate between them and free-living nematodes.

The Baermann isolation technique first described by Baermann (1917) has been employed by a number of workers in connection with hookworm larvae. The writer made critical tests with this method on *Trich.*, *H. cont.* and *Oes. col.* larvae in faeces cultures and in different types of soil and found that it gave very excellent results, comparable to those obtained by Cort et al. (1922*a*), with hookworm larvae. The best results were obtained with the coarsest soils and a small apparatus, using funnels of 6 inches diameter.

The method is, in fact, useful for separating not only infective nematode larvae but also free-living nematodes, rotifers, tardigrades, infusoria and, as has been mentioned before, the first and second larval stages of *Trich.* also passed through.

In carrying out this technique circular pieces of fairly rigid, fine-meshed wire gauze about 3 inches in diameter were placed into the funnels of 6 inches diameter and on the gauze larger circular pieces of linen. The latter should fill the funnel but should not project over its rim, else capillary action causes water to be drawn up and spilt. The linen should be soaked in boiling water or sterilized each time after use, and the rest of the apparatus must be thoroughly washed, else larvae may be retained, especially in the linen, and confuse subsequent tests. The soil, etc., is placed on the linen to a depth of 2-3 cm. If a large amount of material is to be examined, it is better to use a number of funnels rather than to use larger funnels. The stem of the funnel is closed with a short piece of rubber tubing and a strong clamp.

Water at about 40° C. is poured down the side, pulling away the linen slightly, and the funnel filled up from the bottom until the water just covers the material. Other authors state that the level of the water should reach just above the lower surface of the soil (Cort et al., 1922*a*). The writer obtained better results when the water covered the soil, as would seem to be indicated by a statement made by the authors just mentioned: " It is probable that the passage of the larvae from the soil to the water is not a reaction to moisture or to a higher temperature, but is brought about by the active larvae falling through spaces in the soil until they reach the water." When the soil is completely under water, the larvae would probably fall through the spaces between soil particles more easily than when the soil is just wet, especially in a tough clay soil, and there would be less opportunity for larvae to attempt upward migration in the material.

Larvae can also be recovered from grass cut up into small pieces and placed in the funnel, but in this case it is advisable to pour the water directly on to the grass.

The identification of the larvae is important, especially in the case of larvae that have lost their sheaths, as they often do in the soil, which has also been observed by Cort et al. (1922*b*) and Augustine (1922*b*) in the case of hookworm larvae. This is a question of practice and experience. A thorough knowledge of the appearance and typical movements of larvae of the different species concerned must first be gained from studying the larvae obtained from cultures. The size and proportions of the larvae, the shape of the tail and the oesophagus, the small size of the genital primordium and the movements of the larvae are the main characteristics by which they can be differentiated from free-living nematodes and the different species from one another.

Experiments on longevity were made at different times of the year in pots of soil and in plots of ground. Some of them are described here.

Experiments in Pots.

EXPERIMENT 16.

An earthernware pot 12 inches high and 12 inches in diameter at the top was filled with moist black clay soil, popularly called "black turf" and a grass plant placed in the soil. On the 18th November, 1927 a large number of infective larvae of *Trich.*, *H. cont.* and *Oes. col.* and some fresh sheep faeces containing numerous eggs of these species, were spread over the top of the soil. The pot was kept moist by adding water at the top. It stood on the verandah in the shade. On the 28th January, 1928 a small number of larvae of all three species were found on the grass and in the soil. On the 25th April, 1928 only 1 *H. cont.* and 2 *Trich.* larvae could be found on the grass and none in the soil.

EXPERIMENT 17.

Two similar pots, one with red sandy loam and one with "black turf" were placed in aluminium vessels of water. The level of the water was so regulated that the top layers of the soil were just kept moist. Grass was planted in each pot and the pots stood on the verandah in the shade, except for a few hours of sunshine daily in the winter. On the 7th May, 1928 both pots were heavily infected with larvae placed on the soil. From the second month onwards the numbers of larvae decreased considerably. After 4 months, on the 4th September, 1928, a fair number of larvae of all three species were still found, all being in the soil, some at a depth of 3 inches. In the sandy loam the larvae migrated deeper down and in larger numbers than they did in the clay. After five months no more larvae were found in the sandy loam and very few were found in the clay soil, always in the top 1 inch; samples were taken up to 8 inches deep. The last *Oes. col.* and *Trich.* larvae were found in the clay soil on the 25th January, 1929, i.e. after 8½ months, and the last *H. cont.* larvae after 9½ months.

Experiments in Plots.

Plots 20 by 20 feet on level ground, with grass and a few small Acacia shrubs, were fenced in with coarse wire netting and a wall of earth 1 ft. high was made all round to prevent the inflow or outflow of rain water.

EXPERIMENT 18.

Plot I on black clay soil was infected on the 11th November, 1927 with water containing many millions of infective larvae of *Trich.*, *Oes. col.* and *H. cont.* Sheep faeces containing numerous eggs of these worms were also spread on the plot daily up to the 18th November, 1927, when another large batch of larvae in water was added.

On the 28th January, 1928, the conditions being favourable for migration, grass from the plot was examined with negative results. A sample of grass taken from under a shrub on the 30th January, 1928 showed a few *Trich.* larvae and the top 1 inch of soil yielded a few *Trich.* larvae; samples were taken to 6 inches deep. After this no more larvae could be recovered from this plot.

The rapid disappearance of the larvae is explained by the condition of the weather at the time of the experiment. Just before the plot was infected heavy rains had fallen and the ground was sodden. After the infection no rain fell for several weeks and the soil became very dry. In the black clay, which becomes very hot, cracks appear and this accelerates the drying process. The larvae, not being able to migrate very well in this dense soil, are killed in the top layers.

EXPERIMENT 19.

Plot II on sandy loam was heavily infected on the 5th April, 1928. After two months only a few larvae of all three species could be found up to 11 inches deep. The upper 4 inches of the soil were dry. After 6 months no more larvae were found.

EXPERIMENT 20.

On the 28th June, 1929, after a light rain, both plots were heavily infected. The temperature was now fairly low so that the soil would not become hot during the daytime. It was again found that downward migration was more marked in the sandy loam than in the clay soil. On the 23rd September, 1929, 14 days after the first spring rain, 1 *H. cont*, larva was found in the upper 1 inch of the clay plot and a few *Trich.* and *H. cont*, larvae in the upper 1 inch of the sandy loam. Another 2 months later the plots were apparently free of infection.

In all the plot experiments millions of larvae had been used for infection and fairly large quantities of soil were examined. The following conclusions are drawn from these tests:—

Under artificial conditions (in pots), when the soil was kept moist from the bottom and sheltered from the hot sun, *Trich.* and *Oes. col.* larvae lived for $8\frac{1}{2}$ months and *H. cont.* larvae for $9\frac{1}{2}$ months. When watered from the top the larvae died off sooner, probably because the water washed them down into the soil and they were forced to expend more energy in migration.

The larvae migrate more easily in sandy than in clay soil and hence, when other factors are favourable, they die off sooner in the former than in the latter on account of exhaustion.

Under natural conditions the larvae live up to about 3 months in soil. A few weeks of dryness with summer heat suffices to kill the larvae in clay soil. Over the winter more larvae survive in sandy

than in clay soil since they can migrate deeper down for shelter in the former, but on the whole the number of larvae, that survive the winter, is small. *Oes. col.* larvae are less resistant than *Trich.* and *H. cont.* larvae. Tests made during the summer months (not described here) show that continual very favourable conditions for migration cause rapid exhaustion and death of the larvae.

The factors which cause sterilization of the soil are therefore: (1) Exhaustion of the larvae, which do not feed in this stage; (2) desiccation; (3) high temperature; (4) exsheathment, which reduces resistance and (5) possibly the action of sunlight, of bacteria and other soil organisms.

The results are comparable to those of other authors on the larvae of the human hookworms—Augustine (1922*a*, *b* and *c*), (1923*a*, *b*), Payne (1922), Cort et al. (1922*b*), Augustine and Smillie (1926), etc. and on the larvae of other nematodes—Brown (1927), Theiler and Robertson (1915), Veglia (1916), Daubney (1928). The longevity of nematode larvae in soils is undoubtedly much shorter in a warm climate than in a more temperate region.

Thermotropism and Skin Penetration.

Thermotropism.—Khalil (1922) tested the thermotropism of *Ancylostoma* larvae by applying the point of a hot needle to the under surface of a watchglass containing the larvae in water and found that they were attracted by the heat. He also tested other larvae and suggested that positive thermotropism might be a characteristic shared by all larvae that are skin-penetrators. Since then other workers have applied this test to larvae of various species and it has been found that the reaction of the larvae gives no indication as to whether they are skin penetrators or not, as can be seen from the following compilation: —

Author.	*Species.*	*Thermotropism.*	*Skin Penetration.*
Khalil (1922)....	*Ancylostoma duodenale*.....	+	+
	Trich. douglassii.....	+	(?)
	H. cont.....	(−)	—
Cameron (1923).	*Bunostomum trigonocephalum*...	+	—
	Nematodirus spathiger.....	—	—
Goodey (1924)..	*Oes. dentatum*.....	(−)	—
Ortlepp (1925)..	*Triodontophorus tenuicollis*.....	(−)	—
Schwartz (1925)	*Bunostomum phlebotomum*.....	+	—
Baudet (1925)...	*Thrichonema* spp.....	+	—
Morgan (1928)...	*Ostertagia circumcincta*.....	—	—
Fülleborn (1914)	*Strongyloides stercoralis*.....	+	+
Mönnig (this paper)	*Trich.* sp.....	+	—
	Oes. col.....	(−)	—

Remarks: (−) Indicates that the larvae are neither definitely attracted nor repelled by the heat. Beller (1928) states that *Bunostomum trigonocephalum* can infect sheep and goats through the skin; however, in his experiments the skin was shaved and there may have been abrasions. Moreover, the finding of eggs in the faeces of the animals 15 and 17 days after application of the larvae to the skin rather speaks for an accidental oral infection in view of the findings of Cameron (1927), who made repeated tests on skin penetration without success. Sprehn (1925) thinks that *B. phlebotomum* may infect cattle percutaneously, on clinical grounds. On the other hand

Cameron (1927) states that " the hosts of the skin penetrating forms are not grass eaters." This does not hold in the case of Strongyloides, of which the species parasitising the horse (De Blieck and Baudet, 1921) and ruminants (this paper) penetrate the skin of these hosts.

It is, therefore, not feasible to generalize on such reactions or habits of larvae. The larvae of *Oes. col.* are neither definitely attracted nor repelled by a hot point, while the *Trich.* larvae were, in several tests, definitely attracted.

Since it is not always clear how much of the progress of the larvae towards the point is due to their own activity and how much to convection currents caused by the heat, the following test was made.

EXPERIMENT 21.

A piece of glass tubing 15 cm. long and 7 mm. in diameter was filled with water containing a large number of *Trich.* larvae and corked at both ends. A thin copper wire was wound around the middle of the tube and one end was left to project at right angles and horizontally from the tube, which was supported at the ends. The projecting wire was now warmed, but the temperature was not raised as high as to cause visible convection currents in the water—this could be controlled by the movements of small particles of matter in the water. The larvae near the middle of the tube soon became very active and migrated towards the warmest region; the reaction gradually spread outwards and after 15 minutes practically all the larvae had collected at the middle of the tube.

Reaction to stain.—Trich. larvae were placed in a drop of water on a slide, under a coverslip. Methylene blue was allowed to run in under the coverslip. The larvae were watched until the preparation dried up. This was repeated several times. The larvae never cast their sheaths, nor did they absorb the stain. This test does not seem to be of any great value, since it does not differentiate between skin-penetration and others, as was originally believed.

Skin-penetration.—Various tests were made with *Trich., H. cont., Oes. col* and *Str. pap.* larvae. The method of Goodey (1922) was used on several occasions with mixed larvae and also with *Trich.* and *Str. pap.* larvae each separately. The tests were carefully carried out according to the descriptions given by Goodey and others and in every case the water containing the larvae was allowed to evaporate. Larvae from young and from old cultures were employed. The results with *Trich., H. cont.* and *Oes. col.* always showed that these larvae did not penetrate into the skin. In the case of *Str. pap.* penetration was observed only on one occasion, while in the other cases no penetration took place.

A number of tests were also made by placing larvae in water on to the abdominal skin of young guinea-pigs, from which the hair had been clipped (not shaved). The animals were killed after varying periods and the skin and subcutis, lungs, liver, blood, trachea, oesophagus and intestines carefully examined. Again only *Str. pap.* larvae penetrated on one occasion and were found in the skin after 2 hours.

Larvae of *Trich.* and *Str. pap.* were applied to the skin of clean lambs in moist cotton wool, tied to the clipped skin and covered with a bandage and the whole left on for three days, or in other cases the larvae were simply smeared on to the coronary, inguinal and axillary regions. The results of faecal examination seemed to show that infection with *Str. pap.* took place but no infection occurred in the case of *Trich.* Six control lambs showed a slight *Strongyloides* infection, which is difficult to avoid, while the four test lambs showed a heavy infection.

It may, therefore, be concluded that *Trich., H. cont.* and *Oes. col.* larvae are not skin penetrators and that infection takes place per os, as has been demonstrated on numerous occasions. Zawadowsky et al. (1929*a*), however, state that "animals may, evidently, be infected with Trichostrongylidae per os and per cutaneum."

It is remarkable, that *Trichostrongylus orientalis*, a human parasite, is a skin penetrator according to the researches of Koino (1925) and others. Koino, however, states that infection *per os* is much more successful than through the skin and is probably the normal route. Even with oral infection the larvae migrate through the lungs according to this author. Kitamura (according to Goodey, 1925) states that the majority of the larvae penetrate the skin of the mouse in the unsheathed condition, which might indicate that skin penetration is not their most normal route into the host. Goodey found the same to happen in the case of *Uncinaria stenocephala*, which in Fülleborn's (1929) experiments did not penetrate through the intact human skin and which, after infection per os, do usually not migrate through the lungs of the dog.

Strongyloides papillosus undoubtedly differs very much from *S. stercoralis* as far as the mode of infection is concerned. It is evidently not a very vigorous skin penetrator and its normal mode of infection is through the oral route. Sheep can easily be infected by administering the larvae per os in water. In a number of cases where this was done, giving thousands of larvae to each sheep, no lung symptoms were noticed although the animals became heavily infested. It therefore seems as if oral infection does not give rise to migration through the lungs, although this question needs further study. *Strongyloides stercoralis*, according to the researches of Fülleborn (1914) is a skin penetrator and, when administered per os, the large majority of the larvae die from suffocation, only rare ones developing in the stomach wall as orally administrated larvae of the dog hookworms do (Fülleborn, 1926, Yokogawa and Oiso, 1926).

SUMMARY.

1. Methods are described for obtaining infections with single species of worms in lambs.

2. Field experiments showed that in the cold, dry winter development is slow and drying overtakes *Trichostrongylus* eggs and larvae long before they reach the infective stage.

3. Of the pre-infective stages of *Trichostrongylus* spp. only eggs with complete embryos resist drying; some recover after 15 months. The pre-infective stages of *Haemonchus contortus* and *Oesophagostomum columbianum* are all killed by drying.

4. The pre-infective stages of *Trich.* spp. are killed by freezing unless they are sheltered. Low temperatures above freezing point cause slower development. High temperatures (37° C.) cause preliminary rapid development followed by deterioration and death.

5. Eggs of *Oesophagostomum columbianum* remained viable under 3 cm. of water for periods up to 13 days, those of *Haemonchus contortus* and *Trich.* spp. up to 45 days. No eggs developed under the water.

6. Infective larvae of *Trich.* spp., *H. contortus* and *Oes. columbianum* are positively phototropic to mild diffused light.

7. Infective larvae of *Trich.* spp. lost the power of migration when kept in culture jars at 37° C. and died in 12 days.

8. Infective larvae of *Trich.* spp. were able to infect a lamb after having been frozen for 10 days. The larvae lose the power of migration from cultures exposed to 0° C.

9. Infective larvae of *Trich.* spp, revived after having been dry on glass slides for up to $8\frac{1}{2}$ months, while the longest period determined under less favourable conditions for *H. contortus* larvae was $4\frac{1}{2}$ months and for *Oes. columbianum* larvae 3 months. The temperature and moisture content of the air and hence also the time of the year are very important controlling factors. It is the actual degree of dryness of the larvae which determines the results of such tests. On reviving, larvae frequently cast their sheaths. Larvae of all three species, which had been dry for 3 weeks, were able to infect lambs.

10. In tapwater infective *Trich.* larvae lived up to 7 months. In vessels of water with mud at the bottom the larvae of *Trich.*, *H. contortus* and *Oes. columbianum* lived $3\frac{1}{2}$ months in the shade and $2\frac{1}{2}$ months when exposed a few hours daily to the sunlight. The larvae of *Strongyloides papillosus* die very soon under water.

11. The suitability of culture media for migration of larvae is discussed. A technique of making semi-solid faecal cultures is described and the use of sterilized sheep faeces to replace charcoal is recommended. The infective larvae of *Trich.* spp., *H. contortus*, *Oes. columbianum*, *Oes. dentatum* and *Strongyloides papillosus* migrate upwards under suitable conditions.

12. The Baermann isolating technique is suitable for the pre-infective and infective larvae of *Trich.* spp. and was also successfully used for the infective larvae of *H. contortus* and *Oes. columbianum*. Rotifers, tardigrades, free-living nematodes and infusoria were also seen to pass through the apparatus.

13. In sheltered pots of clay soil watered from the bottom infective larvae of *Trich.* spp. and *Oes. columbianum* lived for $8\frac{1}{2}$ months and *H. contortus* larvae for $9\frac{1}{2}$ months. In sandy soil they died off sooner, probably from exhaustion since migration is more extensive. In similar pots of clay soil watered from the top the larvae lived about 4 months.

14. Under natural and favourable conditions the larvae live up to 3 months in the soil. They are rapidly killed by dryness and heat

combined, especially in clay soil. Continual favourable conditions for migration exhaust and kill the larvae in a short time. Exsheathment, which occurs frequently, reduces resistance and aids in destroying the infection in the soil. This exsheathment may occur on revival after drying.

15. Infective larvae of *Trich.* spp. are positively thermotropic but are not skin-penetrators. The larvae of *H. contortus* and *Oes. columbianum* are neither definitely attracted nor repelled by a hot point.

16. *Strongyloides papillosus* is not a strong skin penetrator. Infection per os is the natural route of entry and it seems as if no migration through the lungs takes place. In this respect this species differs considerably from *S. stercoralis*.

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A Note on the Preservation of Engorged Female Ticks.

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Since it is necessary that demonstration specimens should retain their normal appearance as much as possible, and colour, especially in insects, ticks, etc., is usually important as a factor to assist in their recognition, many attempts have been made to find suitable methods of preservation.

The writer has for some time been experimenting to find a method for the preservation of engorged female ticks in their natural colours, as it is absolutely essential to have such specimens for demonstration to students, and there was apparently no known method for this purpose.

A large number of preservative fluid mixtures gave unsatisfactory results. Usually the ticks turned a dark brown colour. It was then attempted to inject various mixtures into the body of the tick by means of a syringe and a fine needle, but shrinkage of the specimens could not be prevented. It was noted, however, that mixtures containing Chloroform tended to produce a lighter colour and, working on this basis, a very satisfactory preservative was found.

A quantity of 4 per cent. Formaldehyde (10 per cent. commercial Formalin), sufficient for the preservation of the specimens, is placed in a tube, a few drops of Chloroform are added and the tube is shaken. If all the Chloroform dissolves more is added and the shaking repeated until the solution is saturated. If there is an excess of Chloroform, the solution is poured off from it. Into the saturated solution of Chloroform in Formalin the live ticks are dropped and the tube firmly stoppered. The ticks die almost immediately and their colour remains unchanged.

The following species of ticks, males and engorged females, have been preserved in this way: *Rhipicephalus evertsi*, *Boophilus decoloratus*, *Amblyomma hebraeum*, *Hyalomma aegyptium* and *Ixodes pilosus*. These specimens are now seven months old and still resemble live specimens in colour.

Since Chloroform slowly decomposes, it was expected at first that the colour of the ticks would alter after a time. Perhaps this may still happen, but the tubes have been opened occasionally and the smell of Chloroform is always strong. If necessary, a drop of Chloroform could be added occasionally.

Although it remains to be seen whether the specimens will retain their colour after a few years, the method seems to be so satisfactory up to the present, that it was thought advisable to publish this note for the benefit of others.

Possibly the method could also be applied to other material, e.g., caterpillars, spiders, etc., with success. The writer has not had the opportunity to investigate in that direction, but it was found that the larvae of *Gastrophilus* spp. from the stomach of the horse did not retain their natural colours.

Anoplostrongylus Ornatus: A Correction.

Under the name *Histiostrongylus ornatus* a nematode was described from the bat *Eptesicus capensis* (see Mönnig, 11th and 12th Rept. Dir. Vet. Educ. and Res., Union of S. Afr., 1927, p. 224). In 1926, while this article was in the press Boulenger (Parasitology, Vol. 18, No. 1, pp. 86-100) separated *Histiostrongylus paradoxus* Travassos, 1918, and *H. tipula* (Van Beneden, 1873) from the genus *Histiostrongylus* and created the genus *Anoplostrongylus* for them. Also in 1927 Schwartz (Proc. U.S. Nat. Mus., Vol. 71, Art. 5, pp. 1-4) described from a "brown bat" a nematode *A. delicatus* which he referred to this genus.

In am indebted to Dr. Schwartz for drawing my attention to these facts and a re-examination of the material has proved that *H. ornatus* should also be referred to this new genus and should therefore be known as *Anoplostrongylus ornatus*.—H. O. Mönnig.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

A Spirurid (*Streptopharagus geosciuri*, sp. nov.) from the Stomach of the Cape Ground Squirrel (*Geosciurus capensis*).

By P. L. LE ROUX, B.Sc., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In July, 1929, the writer collected several nematodes from the stomach of a male Cape ground squirrel. A microscopic examination of these parasites revealed the presence of four specimens (two males and two females) of *Physaloptera capensis* Ortlepp, 1922 and twenty-nine (18 males and 11 females) specimens of a species of *Streptopharagus* Blanc, 1912. Most of the latter are sexually immature. The members of the genus *Physaloptera* were more firmly attached to the gastric mucosa than those of the genus *Streptopharagus*. The members of this genus were found with their anterior extremities buried in the mucosa. The cuticle in the cervical region seems specially modified to this purpose.

Streptopharagus geosciuri, sp. nov.

The males measured vary in length from 12.5 mm. to 16.7 mm. with a maximum diameter of 215μ to 250μ. The corresponding measurements in the females were 24 mm. to 33 mm. and 246μ to 415μ (average for apparently matured specimens 385μ).

The cuticle is transversely striated and is in the region of the oesophagus furnished with low intersected longitudinal ridges formed by a thickened portion of the cuticle between the transverse striations. These thickenings appear like rods. The mouth is bounded by two lateral trilobed lips. The lateral diameter of the cephalic extremity varies from 31μ in the male to 33μ in the female. The buccal cavity continues posteriorly into a tubular pharynx marked with irregular transverse striations. The length of the structure varies with the degree of contraction of the body. The half turn of a spiral displayed about the middle of the pharynx in the known members of this genus is undoubtedly due to contraction of the cephalic extremity. The cuticular cephalic inflation recorded for some members of the genus should probably likewise be attributed to the same agency.

In live specimens of *S. geosciuri* the appearance and the disappearance of the half spiral are readily followed. In only a few cases of the preserved specimens is this half turn of a spiral present and in no single specimen is the cervical cuticle inflated. The pharynx is followed by a long oesophagus measuring 1.7 mm. to 2.1 mm. in the males and 2.1 mm. to 2.6 mm. in the females, and

with a maximum diameter of 96μ to 115μ and 112μ to 141μ in the males and females respectively. At the base of the pharynx the width is 35μ to 45μ in the male and 45μ to 58μ in the female. The cervical papillae are asymmetrically arranged. The left papilla is the more anteriorly placed. Mönnig (1924) figures *Streptopharagus armatus* with the right papilla nearer the anterior end. This is undoubtedly not the case. In *S. geosciuri* the left and the right papillae are situated at intervals of 160μ to 170μ (male), 160μ to 245μ (female) and 320μ to 432μ (male), 352μ to 432μ (female) respectively from the anterior extremity. The nerve ring is situated slightly in front of the left cervical papilla. The excretory pore is approximately on a level with the right cervical papilla. In males it is 294μ to 320μ and in females 320μ to 432μ posteriorly to the cephalic end. The cephalic extremity bears the usual six papillae. The four submedians are well defined while the two medians are rather rudimentary. The examination of the cephalic extremity of a female of *Streptopharagus pigmentatus* (v. Linstow, 1897), Rail., and Henry, 1918 from *Papio porcarius*, Cape Province, confirms Ortlepp's findings that there are two large submedian papillae on each lip, instead of the two groups of three as recorded by Mönnig (1924).

Male Characters.

The posterior extremity has well developed caudal alae and is in preserved specimens strongly coiled, rendering a thorough examination of the ventral surface rather difficult. By allowing specimens to die in cold physiological saline this marked coiling was partly eliminated and allowed of a more careful examination. This examination of unpreserved specimens proved that the presence of the clawlike structures recorded for *Streptopharagus baylisi* Ortlepp 1925 (syn. *Streptopharagus armatus* Blanch of Baylis, 1923), was merely the result of the folding of the ventral cuticle while orientating the caudal extremity under a heavy coverslip so as to obtain a good view of its ventral aspect. These claw-like structures on one side of the cloacal aperture would disappear and reappear on the other side when the coverslip was moved in a reverse direction. It was then evident that what really had happened was that when the coverslip was moved to the right the cuticle on the ventral surface to the left of the anus folds on itself and the thus folded on themselves longitudinal ridges produced the claw-like structures. Their presence in preserved specimens must be due either to the contraction of certain muscles or to the pressure applied for flattening out the bursa. They can no longer be regarded as of any specific generic importance. This observation undoubtedly proves the desirability of examining live or unpreserved specimens whenever possible.

The caudal bursa bears four pairs of preanal and one pair of postanal pedunculated papillae, a single large sessile median papilla on the anterior border of the cloacal aperture, a pair of sessile papillae just behind the anus, and five pairs of small papillae, closely grouped together, on the ventral aspect of the tail. The pair of postanal sessile papillae is somewhat hidden by the cuticular bosses and longitudinal striations in that area. Ortlepp (1925) records their presence in *S. armatus* and in *S. baylisi*.

The presence of a single large sessile preanal papilla has not hitherto been recorded for any of the other members of the genus. Among the *camera lucida* drawings made of helminths during my stay at the London School of Tropical Medicine in 1924, there is one (Fig. 4) of the caudal extremity of a male *Streptopharagus armatus* from *Macacus nemestrinus*. In this drawing there figure a pair of sessile papillae on the posterior lip of the cloacal opening and a single median sessile papilla on the anterior lip of the same aperture. Ortlepp (1925) does not figure this median preanal papilla. Its presence proves the close affinities existing between the genera *Spirocerca* and *Streptopharagus*. Baylis and Daubney (1926) include both these genera in the subfamily Arduenninae Railliet and Henry, 1912, while Yorke and Maplestone (1926) retain them in separate subfamilies. Their retention in separate subfamilies does not seem natural.

The spicules are unequal, the left being 2.23 mm. to 2.62 mm. and the right 416μ to 448μ long. The gubernaculum has a length of 54μ to 64μ.

Female Characters.

The vulva is a rather small circular aperture situated at a distance of 7.5 mm. to 10 mm. from the anterior extremity. The genitalia is of the type as figured by Seurat (1914) for *Spirocerca sanguinolenta* (Rud.). The vagina continues posteriorly to divide into two uteri, one of which turns anteriorly. The eggs in the vagina contain embryoes and measure 35.2μ by 17.6μ. The anus is situated 231μ to 269μ in front of the blunt posterior extremity which carries a pair of caudal papillae.

Remarks.

This species would appear to be closely related to *Streptopharagus numidicus* Seurat, 1917, from *Vulpes cerdo*. Ortlepp (1925) records having collected two partly decomposed specimens of *S. numidicus* from *Gerbillus pygarus* and he gives the length of the left and right spicules as 2.2 mm. and 480μ respectively. This compares well with those of the species described here. Seurat's species is inadequately described, and it is therefore impossible to ascertain with any degree of certainity whether it is identical with the species from *Geosciurus capensis*.

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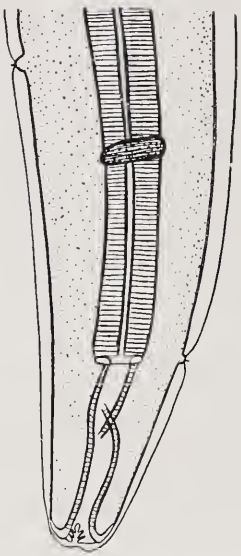

FIG. 1.
Dorsal view, anterior extremity of *S. geosciuri.*

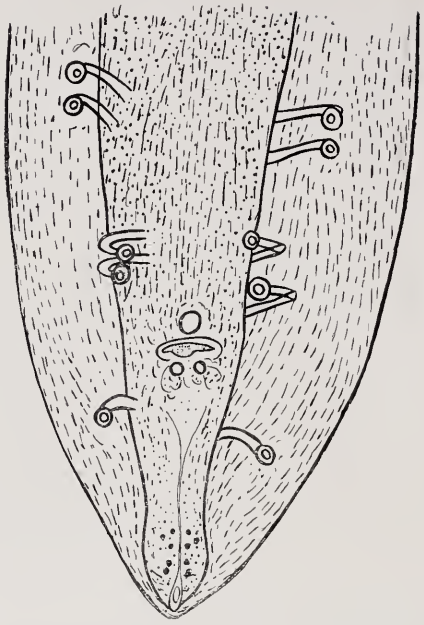

FIG. 2.
Ventral surface, posterior extremity of a male—*S. geosciuri.*

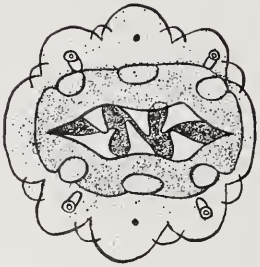

FIG. 3.
Anterior view of the cranial end in *Streptopharagus pigmentatus.*

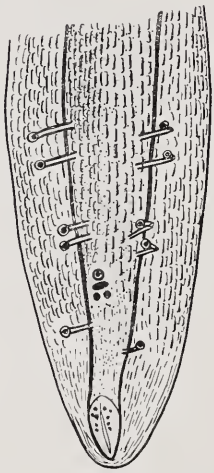

FIG. 4.
Caudal extremity of *Streptopharagus armatus* from *Macacus nemestrinus.*

The Generic Position of *Oxyuris polyoon* von Linstow, 1909, in the Sub-family *Oxyurinae*, Hall, 1916.

By P. L. LE ROUX, B.Sc., M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

In July, 1929, the writer collected and preserved in glycerine alcohol oxyurids from the caecum and colon of a Cape ground squirrel (*Geosciurus capensis*, syn. *Xerus setosus*) shot on a farm in the district of Bloemhof, Transvaal. The examination of specimens cleared in lacto-phenol proved them to be specimens of *Oxyuris polyoon* von Linstow, 1909. This species was originally recovered from the same host at Rehoboth, in the then German South West Africa.

The original description of this worm is so meagre that Hall (1916) expressed the view that it should probably be transferred to the genus *Dermatoxys* Schneider, 1866. Hall was most likely influenced by Seurat's (1915) remarks that von Linstow's species was very closely related to the species *Dermatoxys getula* described by him from the caecum of *Atlantoxerus getula* (syn. *Xerus getulus*) in Morocco. Yorke and Maplestone (1926) list *D. polyoon* (v. Linstow, 1909) as a doubtful species of *Dermatoxys*.

An examination of the specimens collected by the writer proved that von Linstow's species is a member of the genus *Enterobius* Leach, 1853, and is, therefore, re-named *Enterobius polyoon* (v. Linstow, 1909). As synonyms should be cited:—*Oxyuris polyoon* von Linstow, 1909, and *D. polyoon* (von Linstow, 1909), Yorke and Maplestone, 1926.

Morphology of *Enterobius polyoon* (v. Linstow, 1909).

Specific diagnosis, *Enterobius*: Mouth with three prominent lips (one dorsal and two subventral); cuticle inflated anteriorly, and transversely striated; narrow lateral alae arising close behind the nerve ring and extending almost to the posterior extremity in the female; no buccal capsule; oesophagus with a club-shaped anterior portion separated from the oesophageal bulb by a constriction, oesophageal bulb containing a valvular apparatus; excretory pore opening slightly caudal to the posterior limit of the oesophagus, nerve ring just posterior to the cephalic inflation.

Male: Posterior extremity truncated, caudal alae supported anteriorly by a pair of stout pedunculated preanal papillae and posteriorly by a somewhat similar pair arising from a sublateral position close to the caudal extremity of the body, and laterally by a pair of adanal papillae followed by a pair of postanal papillae; another pair of caudal papillae much reduced in size and not supporting the alae,

situated more dorso-laterally than the others; spicule single and relatively long, gubernaculum absent.

Female: Vulva in anterior half of the body; ovejector well developed with the uteri leaving in opposite directions; in gravid females the uteri extending anteriorly half-way up the oesophagus and posterior to the anus; oviparous, eggs with a thick smooth shell.

The Male.

Six of the larger males were selected for the various measurements. They varied in length between 3.4 mm.to 3.7 mm. with a maximum thickness of 277μ to 365μ about the junction of the middle and posterior thirds of the body. The oesophagus measures 836μ to 927μ in length. The anterior club-shaped portion varies between 713μ to 783μ in the different specimens. This portion has a minimum diameter of 38μ to 44μ while the maximum ranges from 70μ to 77μ. The oesophageal bulb measures 128μ to 144μ in length and its width varies between 118μ and 144μ. The lateral diameter of this portion often exceeds its antero-posterior dimension.

The width of the cephalic extremity immediately anterior to the inflation is approximately 51μ while that of the cephalic inflation ranges from 96μ to 115μ. The antero-posterior dimensions of this inflation are 40μ to 112μ on the dorsal and ventral aspects by only 80μ to 95μ on the lateral aspects. In some specimens the dorsal measurement exceeds the ventral but this is reversed in others. Cameron (1929) observes that in *E. vermicularis* the swelling is longer laterally than on the dorsal or ventral aspect.

The excretory pore is situated about 48μ to 64μ behind the posterior limit of the oseophagus while the lateral alae arise at a point situated 243μ to 288μ posterior to the cephalic extremity.

The single testis pass to within 532μ to 380μ of the base of the oesophagus before turning posteriorly. The spicule is heavily chitinised anteriorly and is here furnished with antero-laterally directed lateral appendages. These appendages appear rather fibrous. The spicule, including the appendages, has a length of 262μ to 275μ. The chitinised portion alone measures 214μ to 220μ and has a maximum dorsal-ventral depth of 32μ. In specimens cleared in lacto-phenol the spicule was distorted. It was much swollen about the junction of the posterior and middle third. This distortion was even more marked in a ventral view of the spicule. It would be interesting to know whether the spicules of the other members of this genus are susceptible to similar distortion in lacto-phenol.

A gubernaculum proper is absent but the spicule is evidently guided by a thickening of the dorsal wall of the spicular canal posteriorly. In longitudinal section this thickened portion seems to be composed of material identical with that of the walls of the rectum.

The ventrally curved truncated posterior extremity is furnished with well-developed caudal alae supported by four pairs of pedunculated papillae (one pair preanal, a pair of adanal and two pairs of postanal). The caudal extremity is armed with an additional pair of papillae situated dorsally to the roots of the postanal papillae. This pair of rather slender papillae are by no means conspicuous and is not figured by von Linstow (1909) for *O. polyoon*. That the analogous

pair may be readily missed in the type species of the genus is evident from Fig. 122 D. of Yorke and Maplestone (1926). Lane and Low (1923) figure six pairs of caudal papillae for *E. vermicularis*. Sluiter, Swellengrebel and Ihle (1921) defining the characteristics of the genus *Enterobius* mention that the caudal alae are supported anteriorly by one pair of pedunculated preanal papillae and posteriorly by two pairs of ordinary papillae. They also mention two additional pairs of sessile postanal papillae. In their brief description of the male of *E. vermicularis* they state that there are present on the tail six pairs of small papillae of which the anterior and the posterior are the larger. Cameron (1929) records and figures five pairs of caudal papillae for this worm. He mentions that the pair before the last is very slender. This pair corresponds to the additional pair mentioned above for *E. polyoon*.

The Female.

Females and males are present in approximately equal numbers and the former are not very much larger than the latter. The larger specimens were measured. They vary from 5.25 mm. to 7.5 mm. in length and a maximum thickness of 308μ to 465μ was reached just posterior to the vulva which is situated at a distance of 1.58 mm. to 1.76 mm. from the cephalic extremity. The length of the oesophagus is 1.02 mm. to 1.1 mm. The anterior portion is 851μ to 942μ while the bulbular portion measures 152μ to 160μ. The minimum and the maximum diameters of the prebulbular portion are 48μ and 86μ to 96μ respectively, while the bulbular portion has a width of 165μ to 184μ.

The anterior cuticular inflation is as prominent as in the males, while the lateral alae are readily visible and arise at a point siuated between 246μ and 330μ from the anterior end. The anus is situated between 1.12 mm. and 1.33 mm. in front of the posterior extremity.

The ovejector runs caudally for approximately 1.25 mm. before dividing into the two uteri, one passing anteriorly and the other posteriorly. The uteri are voluminous and closely packed with thick-shelled eggs measuring 64μ by 25μ. The eggs have one side somewhat flattened, causing them to appear asymmetrical in outline when orientated into a certain position. Some of the more matured eggs contain partly developed embryos. The eggs would therefore appear to be deposited in an advanced stage of segmentation. No eggs were recovered from the faecal matter in the caecum or any other part of the alimentary tract.

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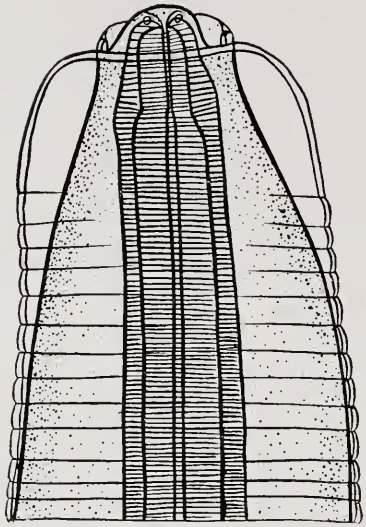


Fig. 1.

Dorsal view of the anterior extremity.

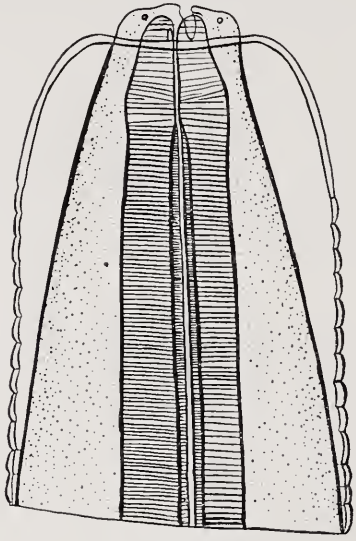
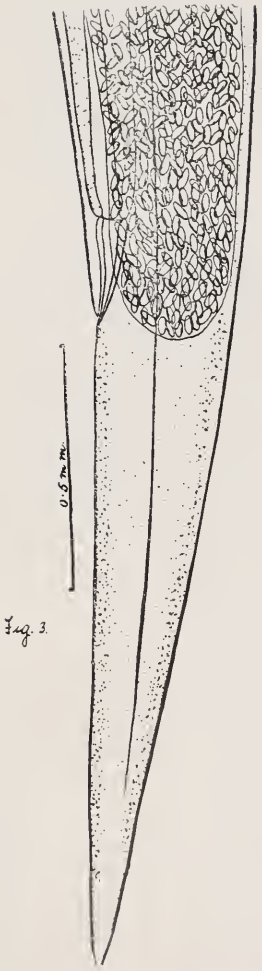
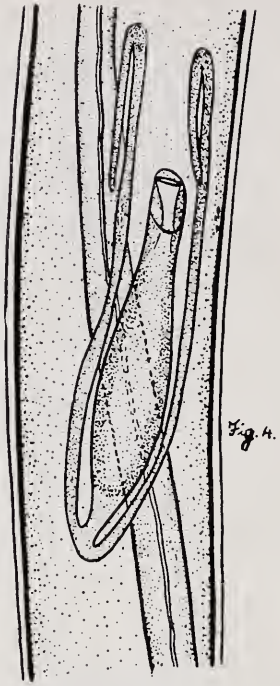


Fig. 2.

Lateral view of the anterior extremity.

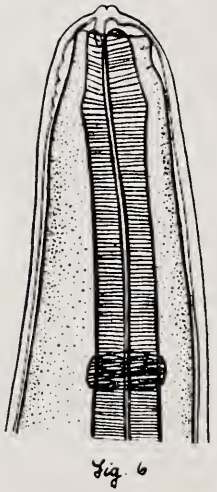


Lateral view of the posterior extremity of the female.



Female genitalia in an immature female.

Immature adult male still within cuticle of the former larval stage.



Anterior extremity of immature adult still within cuticle of the former larval stage.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

An Acuaria (*Acuaria martinagliai* sp. nov.) from a South African Weaver (*Hyphantornis* sp.).

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In December, 1929, Dr. G. Martinaglia visited Johannesburg in connection with an outbreak of Fowl Typhoid amongst a flock of Leghorns. To investigate the possible dissemination of this widespread disease by some of the smaller wild birds, he requested the poultryman to trap some of the Weavers and Cape Sparrows that visit the poultry pens regularly. It is from one of these trapped Weavers that Dr. Martinaglia's assistant, Mr. Grobler, collected two female acuarias. On a personal examination of the gizzard I secured a solitary male. The name *Acuaria martinagliai*, sp. nov. is designated for this worm in honour of my Colleague, Dr. Giovanni Martinaglia, who has contributed much towards our knowledge of the maladies affecting the domestic fowl in South Africa.

The weaver was unfortunately not identified with certainty but is probably *Hyphantornis velatus arundinarius* (Burch).

This is apparently the first record of a member of the genus Acuaria (Bremser, 1877), Cram, 1927, from South Africa.

The chief measurements of the three specimens are appended (see Table 1).

MORPHOLOGY.

Macroscopic Characters.

The fine and hair-like body is in freshly collected specimens practically colourless. These characters camouflage the presence of the parasite on the surfaces exposed after the removal of the horny layer lining the lumen of the gizzard. The females were detected as this lining was separated from the muscular wall of the organ. The male was only discovered on scraping the newly detached surface of the inner tunic with a fine dissecting needle.

Microscopic Characters.—After preservation in boiling glycerine alcohol the specimens were perfectly straight. The caudal extremity of the male was not ventrally curved as is usually the case with *Cheilospirura hamulosa*. The latter is a very common parasite of the muscular portion of the gizzard of South African fowls on free range.

The cuticle bears very fine transverse striations. About the junction of the two portions of the oesophagus they are approximately 3.5μ apart.

The anterior extremity is furnished with a roughly pyramidal-shaped head. The oral aperture is bounded laterally on each side by a conical lip 6-8μ high. The exact limits of these lips can only be ascertained with certainty when the head is viewed either dorsally or ventrally. From a comparison of the descriptions of the different species of *Acuaria* it is evident that some authors are undoubtedly crediting some of the members of the genus with larger lips than they can possibly possess. Most of the recorded species would appear rather inadequately described and figured.

The head is furnished with the usual six head papillae common to most, if not all nematodes. Each lateral aspect of the " pyramid " bears slight pedunculated subdorsal and subventral head papillae towards the angles at the base. In addition to these each also has a rather poorly developed and inconspicuous median head papilla situated about halfway between the apex and the base.

The oral aperture leads into a tubular pharynx with fairly thick wall which appears to be transversely striated. The pharynx is followed by an oesophagus differentiated into an anterior fairly transparent muscular portion and a stouter and more granular posterior portion (the glandular oesophagus).

The inlet to the intestine is guarded by the usual oesophageal valves.

A pair of cordons arise from each oral commissure, and diverge as they cross the dorsal and ventral surfaces of the " pyramid " to proceed true caudally on gaining the subdorsal and subventral median lines of the body. The subventral pair stop slightly short of the posterior limit of the corresponding subdorsal pair. The cordons project but very slightly from the cuticular surface and are not recurrent or anastomosing.

The cervical papillae are rather small and difficult to locate. The left is slightly more posteriorly localized than the right.

The nerve ring is prominent and encircles the muscular oesophagus close to its cephalic end.

Male Characters.—The body attains its maximum width just in front of the caudal bursa which has well developed lateral alae. There are twelve pairs of caudal papillae of which five pairs are definitely preanal, one pair practically adanal and the rest postanal. The postanal pairs are pedunculated and conspicuous, while the others are only very slightly pedunculated or not at all. The spicules are well developed with the right slightly longer but thinner than the left. The single testis extends anteriorly to the base of the oesophagus and turns back.

Female Characters.—The vulva, circular in outline, is localized at the summit of a prominent mound. The dorso-ventral diameter at this point is 200 to 228μ which is also the maximum width of the body. The vagina is anteriorly directed and its first part measures approximately 200μ by 40μ. The ovaries are opposed. The cranial

one extends anteriorly and about halfway up the length of the glandular portion of the oesophagus while the posterior one reaches close up to the rectum before turning cranially. The posterior extremity terminates in a long but rather thick blunt tail, furnished with well-defined subterminally placed caudal papillae.

The uteri and the vagina are packed with thick (4μ) shelled eggs. A large percentage of these eggs are embryonated.

TABLE I.

The Chief Measurements (in Millimetres) of the three specimens.

	Sex.		
	Male.	Females.	
Length of body	8·3	22·5	26·2
Maximum width of body	0·100	0·200	0·228
Anterior end of body to excretory pore	0·280	0·340	0·390
Anterior end of body to cervical papillae—			
Right	0·200	0·228	0·248
Left	0·210	0·256	0·260
Anterior end of body to nerve ring	0·220	0·250	0·260
Anterior end of body to termination of cordons—			
Subventral	0·180	0·348	0·400
Subdorsal	0·260	0·480	0·500
Measurements of pharynx—			
Length	0·180	0·204	0·200
Width	0·017	0·019	0·020
Measurements of first portion of oesophagus—			
Length	0·576	0·592	0·592
Width	0·028	0·040	0·046
Measurements of second portion of oesophagus—			
Length	1·360	2·400	1·680
Width	0·068	0·080	0·116
Anus to posterior extremity of body	0·204	0·280	0·285
Length of spicules—			
Right	0·146	—	—
Left	0·140	—	—
Vulva to posterior extremity of body	—	19·0	12·0
Eggs *in utero* and *in vagina*—			
Length	—	0·040	0·044
Width	—	0·025	0·027

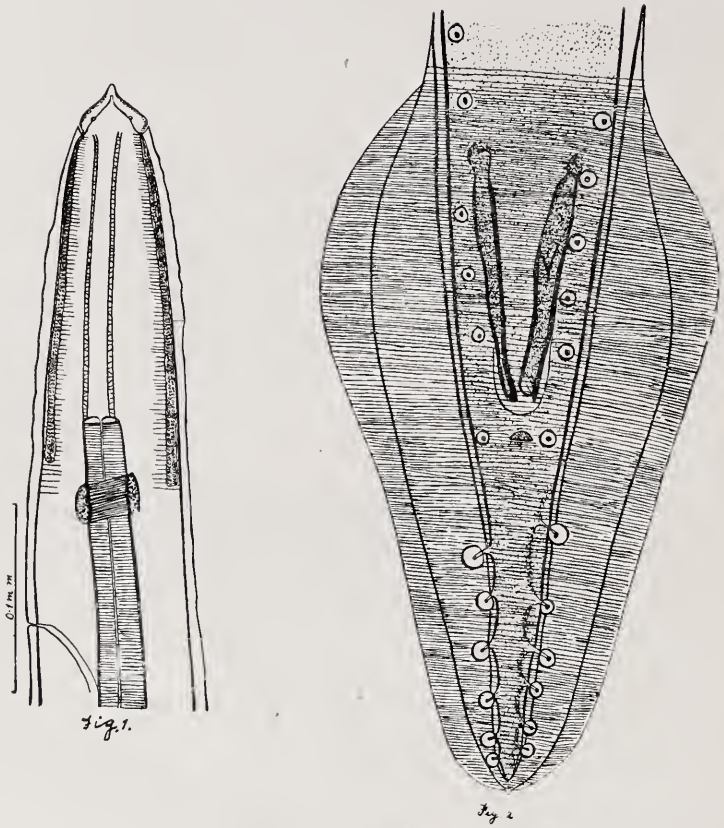


Fig. 1. Lateral view of the anterior extremity, showing the cordons, etc.
Fig. 2. Ventral view of the caudal extremity in male.

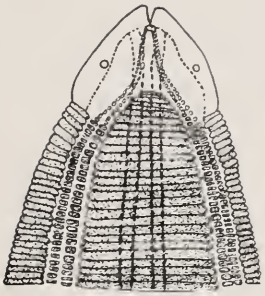


Fig 3

Fig. 4.

Fig. 3. Ventral view of the anterior extremity, showing origin of cordons at oral commissure.
Fig. 4. Caudal extremity of a female.

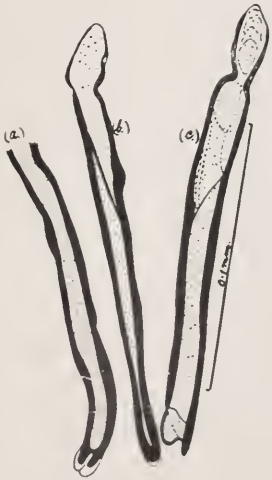


Fig. 5.

Fig. 5 (*a*) Lateral view of right spicule.
Fig. 5 (*b*) Dorsal view of right spicule.
Fig. 5 (*c*) Lateral view of left spicule.

A New Nematode (*Rictularia aethechini, sp. nov.*), a *Physaloptera* and an *Acanthocephala* from the Hedgehog (*Aethechinus frontalis*).

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INTRODUCTION.

In September, 1929, the writer collected four species of helminths from the alimentary tract of a young male hedgehog (*Aethechinus frontalis* syn. *Erinaceus frontalis*) caught close to Hamanskraal Railway Station, Transvaal.

From the stomach were collected a few specimens of a species of *Physaloptera* Rud. These have tentatively been identified as specimens of *Physaloptera dispar*, von Linstow, 1904 (Syn. *Physaloptera incurva*, von Linstow, 1908 recovered from the stomach and intestine of *Erinaceus frontalis* in the Kalahari Desert area.

The small intestine yielded a few tape worms, several Acanthocephala belonging to the genus *Moniliformis* Travassos, 1915 and twelve (four males and eight females) specimens of *Rictularia*, Frölich, 1802. The latter could not be identified with any of the hitherto described members of the genus *Rictularia* and the name *Rictularia aethechini* sp. nov. is proposed for them.

RICTULARIA AETHECHINI sp. nov.

Four males and eight females were collected, examined and measured. The more important measurements are tabulated (see Table I). Some of the specimens were examined alive at the time of their collection.

Macroscopic Appearance and Habits.—The live worms were of a reddish colour and were firmly attached to the intestinal mucosa. On their removal by means of a pair of fine pointed pincettes haemorrhagic spots marked their former sites of attachment. That these parasites suck blood and feed on the cells of the intestinal mucosa are evident. The structure of the buccal cavity is well adapted to those ends. Portions of the intestinal mucosa invariably came away attached to the oral armature, and could not always be removed by shaking the worms vigorously in a tube containing normal physiological saline. On preservation in hot glycerine alcohol the reddish colour of the live specimens was replaced by a dirty white. The females are practically straight while the males appear with

the posterior extremity strongly bent ventrally (Figure 3.) The cephalic end is in both sexes more attenuated than the posterior and the anterior third of the body is slightly ventrally retracted.

Microscopic Characters common to both sexes.—Except for the characters of the buccal capsule, the cephalic and cervical papillae, the oesophagus and the excretory pore, there is little of specific value that is common to both sexes. The cuticle, for practically the entire length of the parasite, is furnished with a row of cuticular appendages along the subventral line on each side. Anteriorly these cuticular appendages, also known as " combs," overlap each other. Posteriorly they are represented as well defined cuticular spines, and are spaced fairly wide apart. The transition from " combs " into " spines " is somewhat gradual. Baylis (1928) observes that too much stress has been laid in many of the descriptions of species of *Rictularia*, upon the distinction between " combs " and " spines." These structures are merely degrees of modification of the same structure. In *R. aethechini* sp. nov. the " combs " consist of a dorso-ventrally flattened free ridge. This ridge increases in height towards its posterior termination where it has a well-marked posterior border forming an angle with the long free lateral border. It is this posterior external angle which becomes more and more acute until we have a definite spine. (Figures 7, 8 and 9.)

Along each side of the attached border of the ridge the cuticle is most characteristically thickened and striated.

Most of the species of *Rictularia* are rather inadequately described as regards the build of the buccal cavity and the characters of the cuticular appendages which are undoubtedly adaptations for progression and anchorage.

The subterminally placed oral aperture faces dorsally and is pentagonal in outline (Figure 2). The buccal capsule is a somewhat complicated structure. Its walls are heavily chinitised and thick (maximum thickness of dorsal, ventral and lateral walls varies from 12μ to 16μ). Its anterior free margin is armed with teeth varying in shape and magnitude. The single mid-ventral tooth (v.b.t. Figure 7) is a continuation of the ventral wall and is long and stout. This tooth has its buccal surface concave both in length and width and it should perhaps be more correctly termed a ventral cutting plate. Mid-laterally the oral aperture on both sides is guarded by a fairly stout lateral buccal tooth (l.b.t. Figure 1). It is triangular in outline and points anteriorly and dorsally.

Between the bases of the above-mentioned teeth the margin of the buccal cavity is armed with smaller teeth which would appear to be homologous with the " row of denticles " described by Hall (1914) for *R. splendida* and by others for different species of *Rictularia*.

In addition to the above-mentioned structures the buccal capsule is characterized by a well-developed *dorsal gutter* arising from the floor and partly from the dorsal wall of the buccal capsule with a length of 50μ to 60μ. In dorsal view it is triangular in outline.

In lateral aspect there appear on the floor of the oesophagus three well-defined projections which will be referred to as oesophageal teeth. They correspond in position to the three sectors of the

oesophagus. In lateral view the subventral teeth appear roughly triangular in outline (subv. o.t., Figure 7), while the *dorsal oesophageal tooth* (Figure 7), rather stout and with rounded tip, is directed anteriorly and ventrally. In a dorsal view the *subventral oesophageal teeth* show prominently.

The cephalic papillae are well-developed and rather characteristic. Each papilla ends in a crescent-shaped structure which appears slightly chinitised. Owing to the distortion of the cephalic extremity the arrangement of the papillae in this genus is somewhat different from that usually met with in nematodes, with the oral opening facing true anteriorly. Their positions except for the behaviour of the subventral pair resemble that of the *Ancylostomidae*. The *subventral cephalic papillae* figure prominently in the anterior half of the cephalic extremity. In dorsal or ventral aspect they appear with a thick common stem but with their extremities free and each ending separately in a crescent-shaped apparently chinitised claw-like structure.

The lateral pair of cephalic papillae is on the anterior level of the buccal cavity while the subdorsal pair is just posterior to the edge of the oral aperture. Hall (1914) observes that head papillae, while doubtless present, are not evident in the male of *R. splendida*. In the female he found them to be ill-developed. Schulz (Figures 17 and 18, 1927) figures for *Rictularia amurensis* Schulz, 1927 prominent cephalic papillae ending in crescent-shaped structures. Baylis (1928*a*) figures for *Rictularia myöbergi* double lateral papillae and neither these nor any of the others have crescent-shaped terminations.

Anteriorly the cervical cuticle is inflated dorsally and ventrally. This inflation is more pronounced dorsally (Figure 1.)

The *oesophagus* is long and consists of an anterior transparent muscular portion followed by a longer and more granular posterior portion.

The *nerve ring* encircles the anterior portion of the oesophagus about its middle.

The cervical papillae are rather short and delicate structures and can be readily overlooked. They are localized about the level of the sixth to seventh cuticular appendage and slightly posterior to the excretory pore which is most difficult to locate owing to it being obscured by the sixth or the seventh cuticular appendage.

The cuticle bears fine transverse striations at intervals of 9μ to 10μ.

Male Characters.—The cuticular appendages extend further caudad than in the females. Each subventral row consists of approximately forty-one "combs" and nine "spines." The so-called transition from "combs" to "spines" is more sudden than in the opposite sex. The first appendage is close to the cephalic extremity. The last pair is usually rudimentary and physiologically defunct.

The single testis is well developed and extends almost to the posterior end of the oesophagus before turning back. There are two rather unequal spicules and rather rudimentary and ventrally situated accessory piece.

The strongly ventrally bent caudal extremity is without alae. The ventral surface is ornamented with cuticular peri-cloacal markings. There are three pairs of pre-anal and five pairs of post-anal sessile papillae. The cloaca opens on the summit of a slight mound. Anterior to the most anterior pair of pre-anal papillae there is in the midventral line a wart-like structure, 100μ in height with an antero-posterior diameter of 160μ. This structure is situated 160μ in front of the anus. The median ventral "combs" of Baylis (1928*b*) or the ventral fans of Hall (1914) were not observed on the ventral surface on any one of the four males of *R. aethechini*.

Female Characters.—The transition from "combs" to "spines" is gradual. There are relatively forty-six combs and twenty-nine spines to each row. The free extremity of the most posteriorly placed spines projects hardly beyond the cuticle. In most cases the last pair is rather rudimentary and merely represented by a thickening of the cuticle. In a young specimen the last pair of spines is anteriorly directed.

The vulva is just posterior to the base of the oesophagus and opens ventrally as a transverse slit opposite the forty-second cuticular appendage. The short and relatively thick vagina continues posteriorly and divides into two *uteri* lying parallel to each other. The *uteri* are packed with eggs. A large percentage of these are embryonated.

The rather short blunt tail ends in a small conical knob. The usual *caudal papillae* are close to the posterior extremity.

Affinities.—About fifteen species of *Rictularia* have hitherto been described from rodents, insectivora and carnivora from various countries. Most of these species are rather inadequately described and figured. *Rictularia aethechini* does not seem to be closely related to any of them.

The sub-family *Rictulariidae* was founded by Hall (1913) because *Rictularia* Frölich, 1802, could not be referred to any of the then established sub-families of the family *Metastrongylidae* Railliet and Henry, 1910. The allocation of genus *Rictularia* to the *Metastrongylidae* cannot be accepted.

Hall (1913) based his conclusion on the structure of the buccal capsule, the oesophagus, the spicules and the female reproductive glands. He, however, observes that it does not possess the normal strongyle bursa and that the cuticular ornamentation and the fact that it is ovoviviparous are not typical conditions in the *Strongylidae*.

Railliet (1916) created the family *Rictulariidae* and included it in the super-family *Spiruroidea* Railliet and Henry, 1915. Yorke and Maplestone (1926) accept Railliet's classification. Baylis and Daubney (1926) express the belief that the genus under consideration should be included in the sub-family *Thelaziinae* of the *Spiruroidae* Oerley (1885). These authors disregard Railliet and Henry's (1915) classification and assign to the super-family *Filarioidea*, Weinland (1858) the families which are grouped in the super-family *Spiruroidea* by Yorke and Maplestone (1926.)

Baylis (1928*a*) still assigns the genus *Rictularia* to the family *Spiruroidae*. The retention of the super-family *Spiruroidae* with the family *Rictularidae* as one of its sub-divisions would undoubtedly simplify rather than complicate the classification of nematodes.

Table I.

Measurements (in millimetres) of Males and Females of *Rictularia aethechini* sp. nov.

	Male.	Female.
Length	5–8·5	15–21
Maximum diameter of body	0·400–0·580	0·536–0·608
Lateral diameter of head	0·132	0·128–0·140
Distance from anterior extremity to 1st comb	0·084–0·096	0·100–0·110
Distance from anterior extremity to nerve ring	0·320–0·440	0·400–0·460
Distance from anterior extremity to excretory pore	0·400–0·480	0·715–0·740
Distance from anterior extremity to base of oesophagus	0·112–0·130	0·132–0·140
Distance from anterior extremity to vulva	—	5·10 –7·500
Length of muscular portion of oesophagus	0·355–0·656	0·624–0·950
Maximum width of muscular portion of oesophagus	—	—
Length of glandular portion of oesophagus	1·920–2·400	3·230–4·200
Maximum width of glandular portion of oesophagus	—	—
Distance from anus to posterior extremity	0·150–0·225	0·144–0·200
Measurements of the eggs in uteri—		
Length	—	0·050–0·060
Width	—	0·032–0·044
Length of spicules—		
Right	0·118–0·132	—
Left	0·190–0·225	—
Length of gubernaculum	0·028–0·034	—

PHYSALOPTERA sp.

The species of *Physaloptera* which have hitherto been recorded from different species of hedge-hogs are:—

1. *Physaloptera clausa* Rud., 1819 from *Erinaceus europaeus*. Seurat's (1917) redescription of the parasite is based on specimens collected from *E. algirus* and *E. deserti* of Northern Africa. This author mentions *Physaloptera dispar* v. Linstow (1904), as a synonym of the type species. Ortlepp (1922) having examined the specimens of *P. clausa*, preserved in the helminthological collection of the Vienna Museum and specimens in the collection of Prof. R. T. Leiper, points out that his observations differ in many respects from those of Seurat. Ortlepp further observes that Schneider (1866) also was probably not dealing with *P. clausa* but a species identical with or closely related to Seurat's specimens.

2. *Physaloptera dispar* v. Linstow (1904) originally described from *E. albiventris* in Nigeria. This species has recently been recorded by Baylis (1928) from *Atelerix spicules* and *A. spinifex* in Nigeria. In this communication Baylis observes that *P. clausa* of Seurat is a synonym of *P. dispar*. He arrived at this conclusion after having re-examined the type-specimens of the latter. He points out that von Linstow's description and figures are inaccurate in several particulars and refers to the discrepancy between Seurat's and von Linstow's descriptions in regard to the length of the spicules. Baylis records that the length of the left spicules varies considerably, ranging from 0.3 to 0.5 mm.

3. *Physaloptera incurva*, von Linstow (1908) recorded as a parasite of *Erinaceus frontalis* (syn. *Aethechinus frontalis*) from the Kalahari region. This species is rather inadequately described by von Linstow as quoted by Ortlepp. Von Linstow's original communication is not available in South Africa. It is quite natural to expect that the specimens described by von Linstow would be identical with those collected by me from the same host.

The Identity of the Transvaal Specimens.—On comparing the measurements (Tables II, III and IV) of the locally collected specimens with the already described species from hedgehogs, it would appear that *P. clausa* of Seurat, *P. dispar* and *P. incurva* and the locally collected specimens are identical, and that a re-examination of *Physaloptera clausa* Rud., may prove *P. dispar* to be identical with it.

The examination of the few males present in my material seems to prove that too much significance should not be attached to: (1) the position of the various pairs of caudal papillae and (2) the character of the post-anal papillae as to whether certain pairs are always pedunculated or sessile. Baylis has shown that the length of the left spicule may vary considerably. Where *camera lucida* drawings of the spicules are given, it would appear essential to have both lateral and dorsal aspects figured. Baylis has rightly referred to the difficulty of determining the precise limits of the spicules in cleared specimens.

Remarks on Certain Structures in the Transvaal Specimens.—It should be stated that the specimens were probably not quite full-grown and this may account for slight differences in certain measurements (e.g. maximum diameter of body).

Ortlepp (1922) observes that he has failed to detect the presence of the additional external papillae recorded by a number of observers as present in the midline of the lip. In the specimens examined by me there is in the midline at the shoulder-like bulging of each lip a small papilla (*median cephalic papilla*) situated in a small depression. These median cephalic papillae are also present in specimens of *Physaloptera capensis*, described by Ortlepp (1922) for *Xerus setosus* (syn. *Geosciurus capensis*), South Africa, and in the specimens of a species of *Physaloptera* collected by the writer on the 28th May, 1929, from the stomach of a native dog and handed to Prof. H. O. Mönnig, who has described and designated them *Physaloptera canis*. The latter does not record the presence of the median cephalic papillae.

In some specimens the loosely attached cuticle forms a prepuce-like collar at the base of the lips. In others this bulging forward of the cuticle over the base of the lips was not evident and in these cases the surface of the cuticle in the region of the base of the lips appear studded with numerous minute elevations (Figure 1). This modification of the surface of the cuticle in this region is undoubtedly an adaptation for facilitating anchorage to the gastric mucosa when the cuticle is extended over the base of the lips.

The chief measurements of three males and five females are tabulated with the measurements of a male and a female of Seurat (1917) The still more immature specimens were not measured.

Table II.

Comparison of the Characters of the Species of *Physaloptera* Recorded from Hedgehogs in Europe and in Africa.

Species	*P. clausa* Rud. 1819.	*P. clausa* Rud. 1819.	*P. dispar* von Linstow, 1904.	*P. incurva*, von Linstow, 1908.	*Physaloptera* sp.
Authors	Ortlepp (1922)	Seurat (1917)	v. Linstow (1904)	v. Linstow (1908)	Le Roux
Hosts	*E. europaeus.*	*E. algirus.* *E. deserti.*	*E. albiventris.*	*A. frontalis.*	*A. frontalis.*
Locality	Europe	North Africa	Nigeria	Kalahari Desert	Transvaal
Length or body ♂	15 to 18 mm.	10·8 to 30 mm.	16·6 mm.	20 mm.	20–22 mm.
♀	30 to 50 mm.	21 to 52·5 mm.	25·0 mm.	47 mm.	33–36 mm.
Maximum breadth of body ♂	825μ–875μ	730μ	530μ	750μ	436–496μ
♀	1·3 to 1·6 mm.	1·350 mm.	950μ	1·58 mm.	672–864μ
Ratio of length of oesophagus to length of body ♂	1 : 5·4 to 1 : 5·6	1 : 4·8	1 : 4·9	1 : 7·6	1 : 5·3 to 1 : 5·9
♀	1 : 7·8 to 1 : 9	1 : 6·9	1 : 6·2	1 : 8	1 : 6 to 1 : 7·2
Ratio of length of muscular oesophagus to total length of organ—					
♂	1 : 7	1 : 8·3	—	—	1 : 7·8 to 1 : 8·8
♀	1 : 8·5	1 : 9·5	—	—	1 : 9·4 to 1 : 9·7
Ration of length of tail to length of body ♂	—	1 : 22	1 : 19	1 : 22	1 : 22 to 1 : 23
♀	1 : 45 to 1 : 55	1 : 54	1 : 26	1 : 235	1 : 63 to 1 : 73
Measurements of the spicules—Right	495 × 50μ	300–325μ	350μ	360μ	300 × 48–52μ
Left	740 × 33μ	275–360μ	620μ	570μ	320–500 × 32–40μ
Measurements of eggs *in uteri*	52 × 38μ	65 × 42μ	57 × 42μ	57 × 39μ	56–60μ × 30–32μ

TABLE III(A).

A comparison of the Measurements (in millimetrs) of a female of *P. clausa* Rud. of Seurat with those of five Specimens of the species of *Physaloptera* from *Aethechinus frontalis*.

Species	*P. Clausa.*	*Physaloptera* sp.				
Authors	Seurat, 1917.	Le Roux.				
Length of body	51·5	33·0	34·0	34·5	35·2	36·4
Maximum width of body	1·350	0·720	0·800	0·672	0·848	0·864
Distance of nerve-ring from anterior end	0·696	0·448	0·512	0·480	0·496	0·516
Distance of cervical papillae from anterior end—						
Right	1·130	0·672	0·816	0·728	0·780	0·992
Left	1·140					
Distance of exeretory pore from anterior end	1·152	0·720	0·806	0·720	0·768	0·720
Distance of vulva from anterior end	8·415	4·100	4·800	5·107	4·300	4·420
Length of muscular oesophagus	0·780	0·496	0·560	0·480	0·544	0·563
Maximum width of muscular oesophagus	—	0·136	0·120	0·138	0·144	0·146
Total length of oesophagus	7·425	4·696	5·310	4·730	5·244	5·425
Maximum diameter of oesaphagus	—	0·320	0·304	0·320	0·336	0·328
Distance of anus from posterior end	0·950	0·480	0·560	0·480	0·560	0·496
Measurement of eggus *in utero*—						
Length	0·065	0·056	0·056	0·058	0·058	0·062
Breadth	0·042	0·030	0·030	0·032	0·032	0·030

TABLE III(B).

A Comparison of the measurements of a male of *P. clausa* Rud. of Seurat with those of the three specimens of a species of *Physaloptera* from *A. frontalis*.

Species	*P. clausa.*	*Physaloptera* sp.		
Authors	Seurat, 1917.	Le Roux.		
Length of body	26·7	20	21	22
Maximum width of body	0·730	0·480	0·496	0·486
Distance of nerve-ring from anterior end	0·530	0·400	0·400	0·400
Distance of cervical papillae from anterior end—				
Right	0·900	0·576	0·562	0·608
Left	0·905			
Distance of exeretory pore from anterior end	0·995	0·592	0·576	0·622
Length of muscular oesophagus	0·600	0·416	0·456	0·435
Maximum diameter of muscular oesophagus	—	0·108	0·102	0·102
Total length of oesophagus	4·820	3·666	3·556	4·135
Maximum diameter of Oesophagus	—	0·224	0·232	0·210
Length of spicules—				
Right	0·300	0·300	0·300	0·300
Left	0·360	0·384	0·320	0·500
Distance of anus from posterior end	1·210	0·880	0·912	0·896

MONILIFORMIS MONILIFORMIS (BREMSER, 1811).

Two species of *Moniliformis* Travassos, 1915 have hitherto been recorded from hedgehogs. Southwell and Macfie (1925) describe *Moniliformis erinacei* from the hedgehog (*Erinaceus europaeus*) at Accra, West Africa. Von Linstow (1904) describes *Echinorhynchus cestodiformis* from the Nigerian hedgehog (*Erinaceus albiventris*.) Van Cleave (1924) having examined the type of von Linstow's species pronounced it to be identical with the type species *Moniliformis moniliformis* (Bremser 1877), which has *Erinaceus europaeus* from Europe as its type host. Southwell and Macfie examined specimens of the type species collected from *Mus rattus* and *Mus norvegicus* at Liverpool, West Africa (Freetown and Accra), South America (Manaos), and Australia (Townsville), from *Cricetomys gambianus* at Accra, and one specimen from *Homo sapiens* at British Honduras. Subramanian (1927) describes *Moniliformis spiralis* from the intestine of *Nesopia bengalensis* at Rangoon.

The measurements of the species from the South African hedgehog are tabulated with those of others (see Table IV). From a study of these measurements it is evident that the specimens from the local hedgehog have certain measurements in common with the type species and others in common with *Moniliformis erinacei*, Southwell and Macfie (1925), of which only a solitary pair (male and female) was examined by the authors.

It is evident from the description of *M. moniliformis* that the measurements of the same structure vary within very wide limits. The measurements quoted for the species from the local hedgehog are of specimens preserved in 10 per cent. formalin and cleared in lacto-phenol. Specimens were dissected for the determination of the exact length of the lemnisci, which differed in length even in the same specimen. They reach the greatest length in the more matured individuals.

Southwell and Macfie (1925) list as synonyms of *Moniliformis moniliformis* the species:—

1. *Echinorhynchus moniliformis*, Bremser, 1815.
2. *Gigantorhynchus moniliformis* of Railliet *et al.*
3. *Hormorhynchus moniliformis* of Ward, 1917.
4. *Echinorhynchus cestodiformis*, v. Linstow, 1904.
5. *Gigantorhynchus cestodiformis* of Porta.
6. *Moniliformis cestodiformis* of Travassos.

Baylis (1929) records *M. moniliformis* as a parasite of rats, mice and squirrels and occasionally of man and dog. As probable synonyms he lists the species:—

1. *Echinorrhynchus* grassi Railliet, 1893 from a dog in Sicily.
2. *Echinorrhynchus canis* Porta, 1914 from a dog in Brazil.
3. *Echinorrhynchus hominis* Leuchart, 1876 from a human in Prague.

The differences between the specimens of *Moniliformis* collected from the various mammals are so insignificant that they should probably all be regarded as members of the same species.

Table IV.

A Comparison of the Characters and Measurements (in Millimetres) of Specimens of *Moniliformis* from Mammals.

Species	*M. spiralis*	*M. moniliformis*	*M. erinacei*	*M. cestodiformis*	*M. moniliformis*	*M. moniliformis*
Authors	Subramanian (1927)	Southwell and Macfie (1925)	Southwell and Macfie (1925)	v. Linstow (1904)	Baylis (1929)	Le Roux
Hosts	*Nesokia bengalensis*	*Homo Sapiens, Mus rattus, M. norvegicus, Cricetomys gambianus*	*Erinaceus europaeus*	*Erinaceus albiventris*	Man, dog, rats, mice, squirrels	*Aethechinus frontalis* (Syn. *Erinaceus frontalis*)
Length of body ♂	33–44	5·5–86	85	115	40–80	65–90
♀	46	7 –239	110		70–110	100–140
Maximum breadth of body ♂	0·78	—	1·6	2·17	1–7·5	1·7–2·0
♀			1·5			1·6–1·8
Measurements of lemnisci	3·71–3·05	2·40–8·76	7–8 × 0·2	1·70	—	10–22 × 0·16–0·40
Measurements of proboscis	0·21–0·45 × 0·12–0·19	0·50–0·67 × 0·2	0·40–0·50 × 0·20	0·47 × 0·20	—	0·64–0·96 × 0·32–0·40
Measurements of testes	1·25–1·54 × 0·29–0·39	0·201–4·0 × 0·120–0·96	5·0 × 1·3	—	—	2·60–4·20 × 0·8–1·10
Measurements of eggs	0·084–0·092 × 0·035–0·046	0·109–0·137 × 0·057–0·063	0·092 0·051	0·085 0·049	0·124–0·127 × 0·071–0·074	0·102 × 0·055 to 0·124 × 0·064
Number of antero-posterior rows of hooks	10–13	12–16	18	14	14	18
Number of hooks in each row	6–7	10–12	7–8	8	15	9

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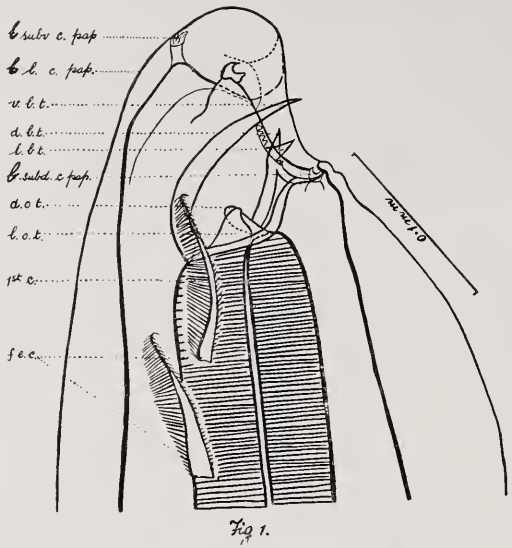


Fig. 1. Lateral view of the anterior extremity. *R. aethechini.*

d.b.t.=dorsal buccal tooth; d.o.t.=dorsal oesophageal tooth; l.b.t.=lateral buccal tooth; l.l.c. pap.=left lateral cephalic papilla; l.o.t.=lateral oesophageal tooth; l. subv. c. pap.=left subventral cephalic papilla; l. subd. c. pap.=left subdorsal cephalic papilla.

Fig. 2. Dorsal view of the anterior extremity. *R. aethechini.*

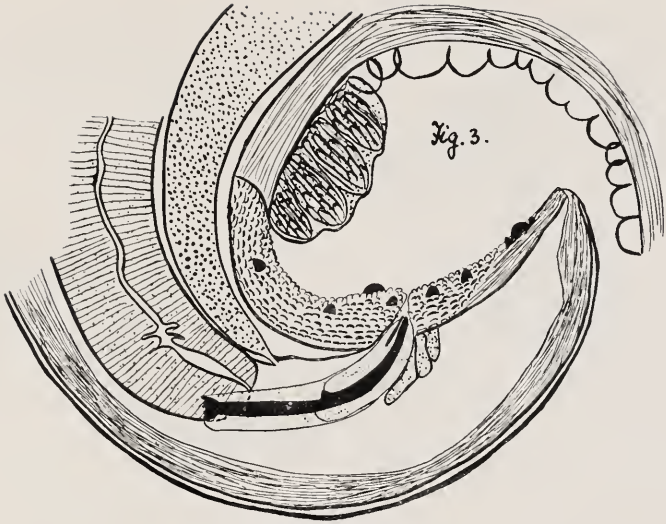


Fig. 3. Posterior extremity of the male. *R. aethechini.*

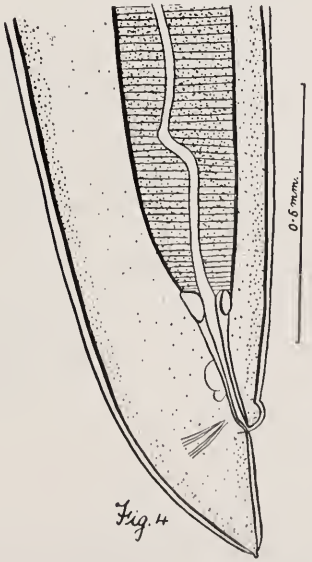
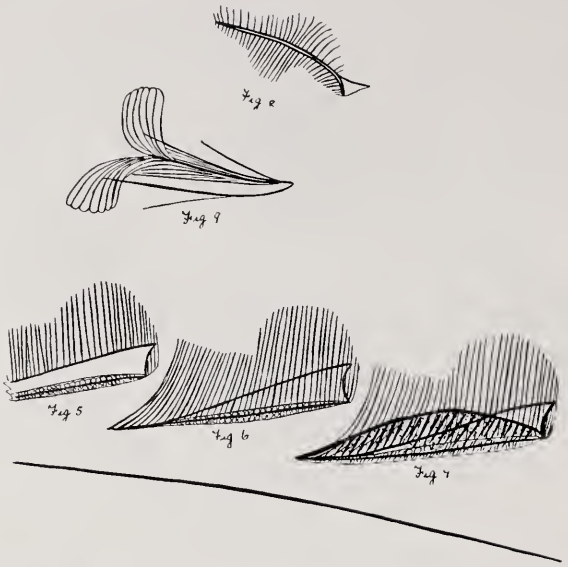


Fig. 4. Posterior extremity of the female. *R. aethechini.*



Figs. 5-9. Differently shaped cuticular appendages. *R. aethechini.*

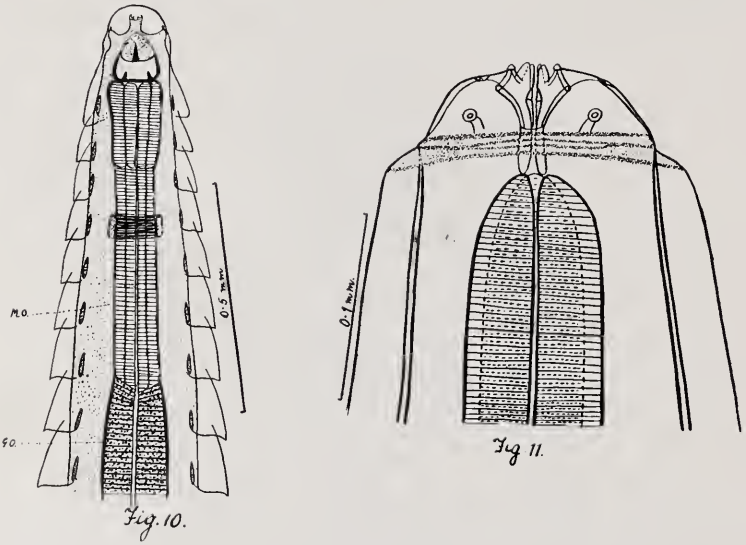
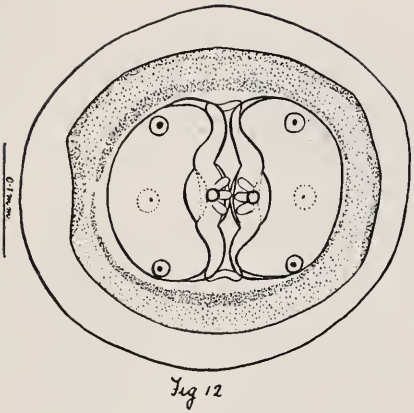


Fig. 10. Dorsal view of the anterior extremity showing the cuticular appendages (combs) overlapping. *R. aethechini.*

M.O. = Muscular oesophagus. G.O. = Glandular oesophagus.

Fig. 11. Laterial view of anterior extremity. *Physaloptera* sp.



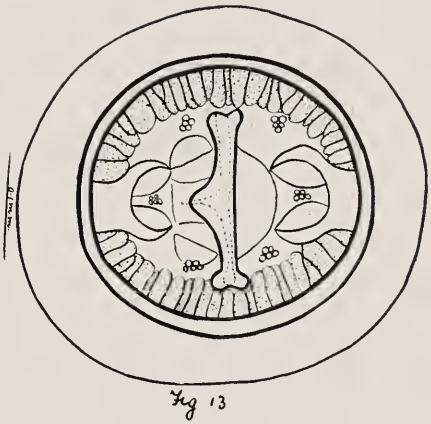


Fig. 12. Anterior of the cephalic extremity. *Physaloptera* sp.

Fig. 13. Transverse section through cephalic extremity just posterior to the lips. *Physaloptera* sp.

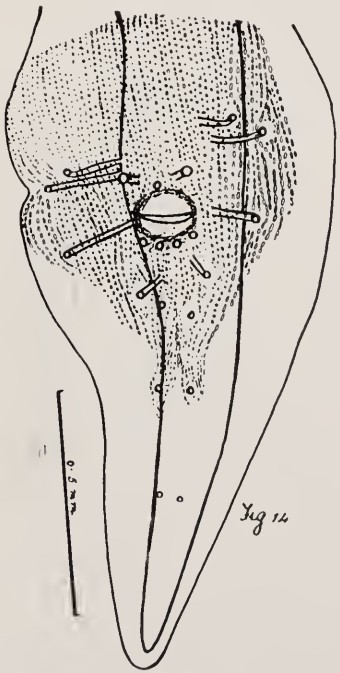


Fig. 14. Ventral view of the candal extremity of a male. *Physaloptera* sp.

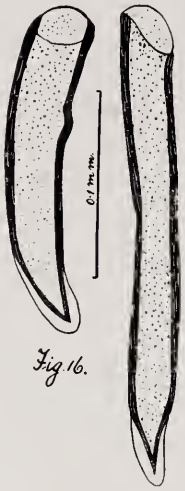


Fig. 15. Lateral view of the caudal extremity of a female. *Physaloptera* sp.
Fig. 16. Left and right spicules. *Physaloptera* sp.

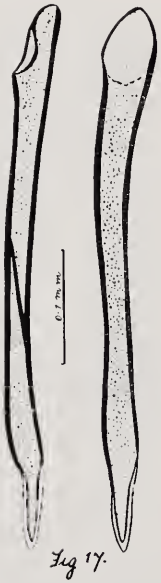


Fig. 17. The same left spicules in different views. *Physaloptera* sp.
Fig. 18. Right spicules from two males. *Physaloptera* sp.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

On Two New Helminths from the Abomasum of the Bushbuck in Zululand, Natal.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In August, 1929, the writer spent a week in Zululand with a ranger who is shooting game under the direction of Mr. R. H. Harris, Director of Operations, Tsetse Fly Control. During this short stay there were facilities for the collection of parasites from buck shot in an area south of the White Umfolozi River. Most of the animals examined were relatively free of helminths, for from some, only three or four specimens were obained, while the majority harboured no macroscopic helminths whatever.

The bushbuck (*Tragelaphus sylvaticus*) yielded two species new to science, these being from the abomasum. For the one species, which is a member of the genus *Ostertagia* Ransom 1907, the name *Osteragia harrisi*, sp. nov. is proposed in honour of Mr. Harris who assisted me in many ways. The females of the other species resemble those of *H. contortus* so closely that only a microscopic examination allows of a differentiation from members of the genera *Haemonchus* Cobb, 1898, and *Mecistocirrus* (Railliet and Henry, 1912), Neveu-Lemaire, 1914. Since the specimens of this species could not be allocated to any known genera of the sub-family *Trichostrongylidae* Leiper, 1908, the name *Ashworthius pattoni* gen. et sp. nov. is proposed in honour of Prof. J. H. Ashworth and Dr. W. S. Patton, my past teachers of Parasitology at the University of Edinburgh. In addition to these two species the host also harboured specimens of *Haemonchus vegliai* Le Roux, 1929, which has the Cape Kudu (*Strepsiceros strepsiceros* as type host and the Transvaal as type locality. Curson (1928) does not list any helminths from the bushbuck in Zululand; but Mönnig (1928) mentions *Cordophilus sagitta* and *Oesophagostomum columbianum* as parasiting this antelope.

It is indeed a great pity that adequate provision could not be made for the collection of helminths from the wild ruminants now being shot outside the boundaries of the old Umfolozi Game Reserve, where the unique Southern White Rhinoceros is also to be found. It would have been of value to know exactly the degree to which game may be infested with ecto- and endo-parasites.

O'Roke (1927) has sounded a warning regarding parasitism and fauna conservatism. He shows that game protection societies are primarily concerned with the preservation of wild animals against gun-powder and lead, but that very little attention is paid to the vital requirements of the animals or their protection against parasites. He adds: " Food and water, shelter and cover, and protection from enemies are the factors usually considered in planning for

the conservation of wild life. The important but little understood questions of parasitism are beginning to command the attention of naturalists and biologists. The parasitologist can render valuable service to game commissions and wild life institutes through his knowledge of the ways of parasites."

ASHWORTHIUS gen. nov.

Generic Diagnosis.—Trichostrongylinae: Body filiform, head relatively large, buccal capsule rudimentary, buccal cavity small and armed with a stout tooth originating from the dorsal sector of the oesophagus; cephalic papillae present, cervical papillae prominent. *Male*: copulatory bursa with well developed lateral lobes, and a prominent dorsal lobe; bursal formula:—ventro-ventral and latero-ventral arise from a fairly long common trunk; ventro-ventral about half the length and thickness of the latero-ventral; extero-lateral ray is the longest and best developed ray in the lateral lobe and arise in common with the medio-lateral ray from a well developed common trunk which in turn shares a very stout trunk in common with the postero-lateral ray; externo-dorsal ray long but slender and lies parallel to the postero-lateral which diverges distally from the other lateral rays; dorsal ray well developed, terminating in four digits of which the inner two are the best developed. Spicules relatively short, with crests and protuberances (Figures 5 and 6.) Gubernaculum apparently absent. *Female*: Vulva in the posterior half of the body. Eggs segmenting when oviposited.

ASHWORTHIUS PATTONI, gen. et. sp. nov.

One male and five females were collected, preserved in glycerine alcohol and examined, and measured after decolourising in lactophenol. The chief measurements of the male and females are shown in Table I.

Characters common to Male and Female.—The buccal cavity is better developed than in either *Haemonchus* Cobb, 1898 or *Mecistocirrus* (Railliet and Henry, 1912), Neveu-Lemaire, 1914, and appears to be furnished with an extremely poorly developed buccal capsule (Figures 1 and 2). The buccal cavity faces slightly dorsally and is armed with a rather stout buccal lancet bent at right angles on itself and with the sharply pointed free extremity directed dorsally and projecting beyond the edge of the buccal cavity. This buccal lancet, perhaps more correctly termed dorsal gutter, measures approximately 23-25μ along its convex border. It is attached to the dorsal half of the oesophagus by means of two roots, triangular in outline in a dorsal view, but roughly rectangular in a lateral view. After attaining a height of 16μ the buccal lancet bends sharply dorsally. This dorsally pointing portion measures 8-10μ in length. The tooth has a maximum lateral diameter of 10μ. Along the ventral border runs a groove into which the dorsal cephalic gland opens. The head is studded with the usual six cephalic papillae. The median head papillae are slightly more anteriorly situated than the others. Freshly collected specimens are of a darker colour than the common abomasal worms of sheep and cattle. The cuticle bears fine cross striations. Longitudinal striations, as recorded for other Trichostrongylidae, are present.

Male Characters.—The copulatory bursa (Figure 3), consists of two prominent lateral lobes and a well developed symmetrical dorsal lobe measuring 626μ by 180μ. The dorsal ray supporting the dorsal lobe has a strongly developed stem measuring 144μ in length and divides posteriorly into two branches, each of which bifurcates almost immediately into a short, stout inner branch and a less strongly developed outer branch. The approximate arrangement of the rays supporting the lateral lobes are as drawn (Figure 4). The inner surfaces of the lateral lobes are furnished with bosses not unlike those met with in *H. contortus* and *M. digitatus*. The free borders of the lateral lobes are crenated. The prebursal papillae, rather short and inconspicuous, are situated about 156μ in front of the bursa. Just anterior to them the cuticle is inflated laterally. The genital cone (Figure 7) is well developed with a rounded caudal extremity. In lateral view it bears a prominent swelling on its ventral surface. The genital appendages are well developed. The outline of the spicules varies somewhat when viewed dorsally, laterally or medially. In dorsal and ventral views they appear to be furnished with lateral projections at a point about 48μ from the posterior extremity which consists of two parts slightly overlapping each other and expanded ventrally. A gubernaculum appears absent.

Female Characters.—Macroscopically the females would be mistaken for specimens of *H. contortus*. The ovaries are twisted round the intestine and produce the barber's pole appearance recorded as characteristic for the wireworm of sheep. The arrangement of the genitalia is as in the members of the genus *Haemonchus* except for the absence of a linguiform process or cuticular swellings (Figure 8) in the neighbourhood of the vulva, which takes the form of a transverse slit. Posteriorly the body terminates in an acutely pointed tail (Figure 9).

Type host: Adult male bushbuck (*Tragelaphus sylvaticus*). *Habitat*: Abomasum. *Type locality*: Zululand, south bank of the White Umfolosi River. *Type specimens and co-types*: Will be deposited in the Helminthological collection at Onderstepoort.

TABLE I.

Measurements (in Millimetres) of *Ashworthius pattoni* gen. et sp. nov.

	Male.	*Females.*
Length	17·0	20·0 –24·0
Lateral width of head	0·050	0·045– 0·052
Thickness at level of cervical papillae	0·115	0·112– 0·138
Thickness at base of oesophagus	0·190	—
Thickness in front of copulatory bursa	0·282	—
Thickness at level of vulva	—	0·308– 0·354
Length of oesophagus	1·39	1·39 – 1·62
Width of oesophagus anteriorly	0·028	0·035– 0·037
Minimum width of oesophagus	0·022	0·028– 0·032
Maximum width of oesophagus	0·144	0·160– 0·170
Anterior end to cervical papillae	0·385	0·324– 0·462
Anterior end to excretory pore	0·278	0·262– 0·338
Length of spicules	0·400	—
Vulva to anus	—	3·08 3·88
Anus to posterior end	—	0·539– 0·554
Caudal papillae to posterior end	—	0·162 - 0·200
Measurements of ova *in utero*	—	0·094–0·098 x 0·048– 0·050

OSTERTAGIA HARRISI sp. nov.

Two males (one damaged) and seven females were collected from a portion of abomasum and preserved in 10 per cent. formalin. Having been kept in cold formalin the specimens are much coiled.

Characters common to both Sexes.—The opening of the small mouth cavity faces anteriorly. The cuticle of the head is slightly dilated (Figure 10), and followed by a constriction. The cuticle is marked by rather fine cross striations and more prominent longitudinal ridges which, under high magnification, have a beaded appearance as defined by Ransom (1907) for the genus *Ostertagia*. These longitudinal striations decrease to a number as the cephalic end of the worm is approached and only half their number proceed anteriorly beyond the cervical papillae. The sharply pointed cervical papillae are not placed laterally, but slightly nearer the mid-ventral line. They are well developed, and appear to be chitinised. The oesophagus is of the usual *Ostertagia* type and the cervical nerve ganglion is situated a little in front of the cervical papillae.

Male Characters.—The copulatory bursa (Figure 11) is of the usual type and about twice as broad as long. The arrangement of the rays supporting the lobes of the bursa are as figures (Figure 11). The spicules are truncated at the tip and appear approximately as drawn [Figure 12 (*a-c*)].

Female Characters.—The vulva is a transverse slit covered with or without a prominent cuticular flap (see Figure 16). The anus is close to the tip of the tail which in all specimens was curled ventrally as figured. The tip of the tail is inflated. The muscular parts of the ovejectors are well developed. Eggs were only present in the anterior uterus. The posterior half of the genitalia contains no eggs and appears atrophied (Figure 13.)

Type host—a young bushbuck ram. *Habitat*—abomasum. *Locality*—Zululand, on the south bank of the White Umfolozi River. *Type specimens and co-types*—Will be deposited in the Helminothological collection at Onderstepoort.

TABLE II.

Measurements (in Millimetres) of *Ostertagia harrisi* sp. nov.

	Males.	*Females.*
Length	3·8	4·6 – 6·3
Lateral diameter of head	0·016	0·016– 0·017
Thickness in front of bursa	0·89	
Thickness at vulva	—	0·091– 0·114
Thickness at anus	—	0·038– 0·042
Thickness at base of oesophagus	0·057	0·055– 0·070
Length of oesophagus	0·448	0·450– 0·520
Maximum diameter of oesophagus	0·032	0·032– 0·040
Anterior end to cervical papillae	0·175	0·200– 0·230
Anterior end to excretory pore	0·169	0·195– 0·225
Vulva to anus	—	0·380– 0·415
Anus to posterior end	—	0·096– 0·112
Length of spicules	0·155–0·160	
Measurements of eggs *in utero*	—	(0·070 x 0·044) – (0·083 x 0·048)

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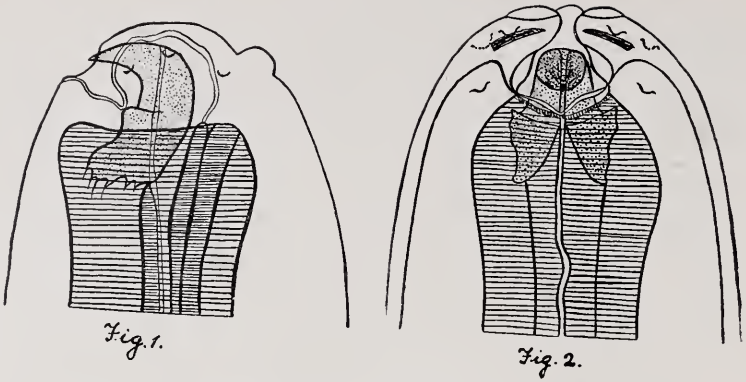


Fig. 1. Lateral view of cephalic extremity. *A. pattoni* sp. nov.
Fig. 2. Dorsal view of cephalic extremity. *A. pattoni* sp. nov.

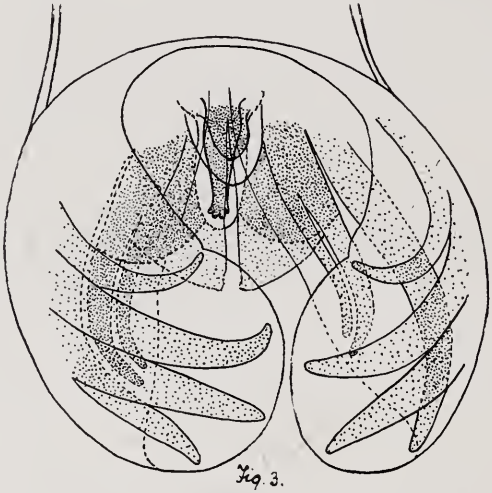
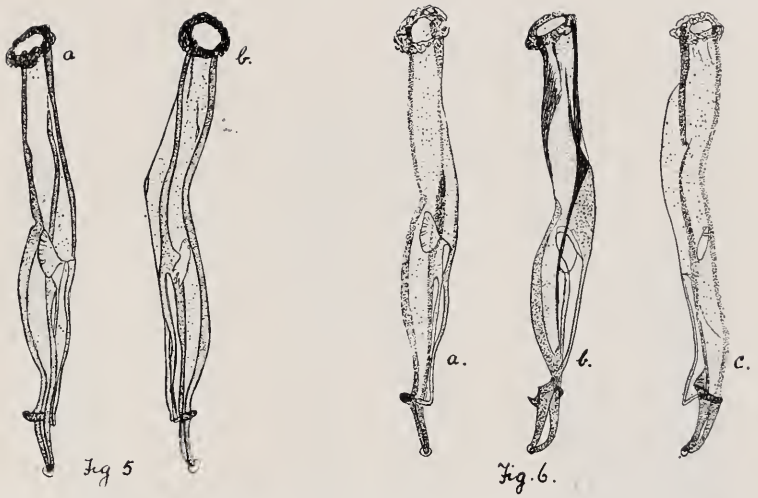


Fig. 3. Ventral aspect of caudal end of male. *A. pattoni* sp. nov.

Fig. 4. Ventral aspect of left lobe of copulatory bursa. *A. pattoni* sp. nov.



Figs. 5 and 6. Various aspects of the spicules. 5*a* and 6*a* left spicule. 5*b* and 6*c* right spicule. *A. pattoni* sp. nov.

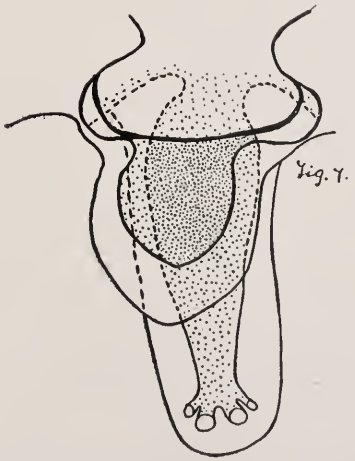


Fig. 7. Ventral view of genital cone and dorsal lobe of bursa. *A. pattoni* sp. nov.

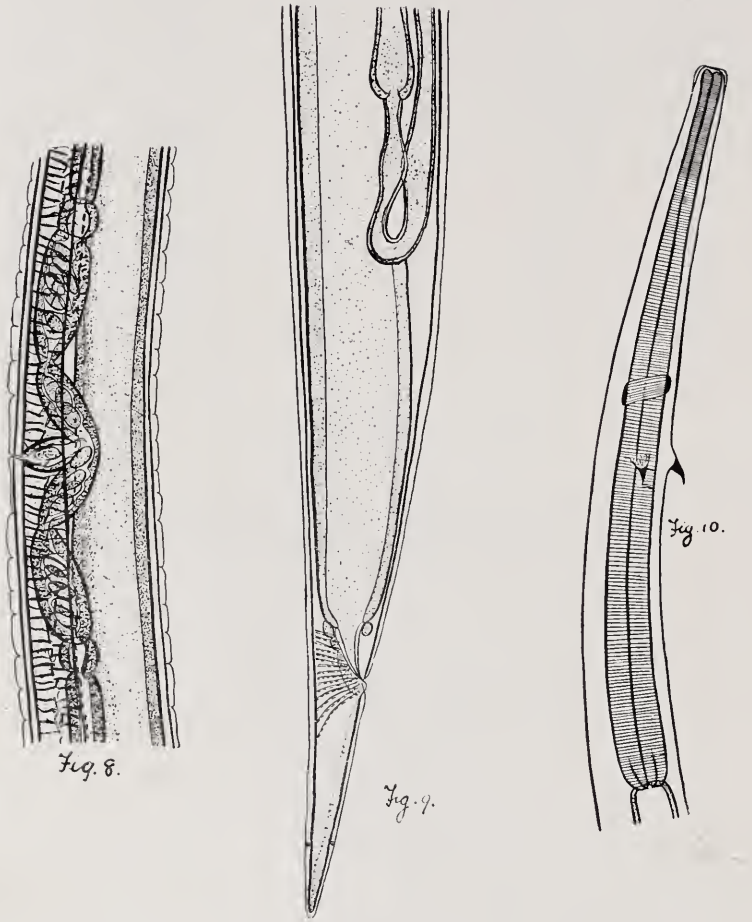


Fig. 8. Portion of female, showing parts of genitalia. *A. pattoni* sp. nov
Fig. 9. Posterior end of female. *A. pattoni* sp. nov.
Fig. 10. Anterior end of a female. *O. harrisi* sp. nov.

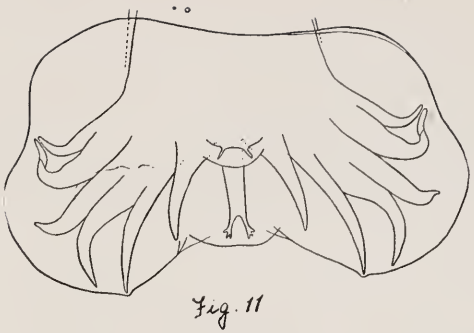


Fig. 11. Ventral aspect of the copulatory bursa. *O. harrisi* sp. nov.

Fig. 12. *a*. Lateral view of left spicule and gubernaculum. *O. harrisi* sp. nov.
b. Right spicule.
c. Left spicule.
Fig. 13. Posterior extremity of female. *O. harrisi* sp. nov.

A Preliminary Communication on the Life Cycle of *Cotylophoron Cotylophorum* and its Pathogenicity for Sheep and Cattle.*

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

Within the last few months I had the opportunity of investigating two serious outbreaks of paramphistomiasis amongst sheep in the Orange Free State. These investigations and subsequent research proved that the immature stages of the paramphistome *Cotylophoron cotylophorum* (Fischoeder, 1901), Stiles and Goldberger, 1910, is most pathogenic for sheep and that the paramphistomes can no longer be regarded as the innoxious parasites that they were once considered to be.

The Outbreaks Investigated.

The first farm to be visited was " Breezy Brae " near Arlington. The owner here lost about 30 per cent. from a flock of approximately 275. These sheep were running in a camp together with some native-owned cattle and had to drink from a rather muddy natural pool. The muddiness of the pool must be attributed to the presence of the cattle that wade well into the water when coming to drink. The examination of this pool proved that it was harbouring thousands of snails, members of a species of *Bulinus*. The species is probably identical with *Bulinus schakoi* (syn. *Isidora schakoi*) from which Cawston (1918), described *Cercaria frondosa*. Faust's (1919) description of this cercaria holds for the cercariae which proved to be the larval forms of *Cotylophoron cotylophorum*.

The sheep on autopsy had no adult or immature paramphistomes in the rumen but sexually immature forms were present in the abomasum and the rest of the alimentary tract posterior to it. They were most numerous in the duodenum and some were also passed in the soft black faeces. The affected animals were at the time of my visit suffering from a most foetid diarrhoea.

Prior to my arrival at the farm the sheep had been removed for some days from the camp where they took ill, because the water was on account of its turbidity regarded as unsuitable for consumption and declared to be the cause of the trouble. The presence of snails was evidently overlooked.

Six animals were bought and railed to Onderstepoort for further investigation. On their arrival at the institute one was killed and immature paramphistomes were recovered only from the abomasum

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and the intestines. To ascertain whether these trematodes were normal parasites of sheep and goats, portions of the duodenum, with parasites attached, were fed to two boer goat kids and three merino lambs born in the small experimental camps on this station and therefore free of infection. One of the kids was killed a week later and sexually immature paramphistomes were recovered from its abomasum and duodenum. One of the lambs, killed a fortnight after the kid, was found to harbour specimens in the rumen and a very few in the abomasum and duodenum. Another lamb killed six weeks after infection harboured specimens only in the rumen. One of the originally introduced sheep was killed on the same day and thousands of specimens were recovered only from the rumen where they were found attached, and closely crowded together.

Five of the sheep were bled for the determination of the blood calcium and phosphorus, and the analysis showed that the calcium content of the blood was much reduced. This reduction in the amount of the blood calcium probably accounts for the presence of the large quantities of sand observed in the abomasum and passing into the duodenum of the affected sheep. The farmers, however, assured me that the first animals that died had no sand in them and there is no reason to disbelieve them. I have often observed sand and other foreign material (coal cinders, granite gravel, small pebbles, etc.) in the rumen and abomasum of sheep suffering from anaemia due to haemonchosis and fascioliasis and in both these diseases the amount of calcium in the blood was appreciably reduced below normal. The blood of the sheep suffering from paramphistomiasis showed a further fall of blood calcium subsequent to the administration of from 4 to 8 c.c. carbon tetrachloride.

The history of the second outbreak investigated is interesting in that cattle seem to have infected the snails. Mr. Frank Hobbs of the farm " Haldon," Petrus Steyn, lost approximately 50 per cent. of a flock of 300 sheep turned into a camp in May when he removed the cattle that had run there since December, 1928. The sheep showed a loss of condition after having been in the camp for about sixteen days. He lost a few and thought nothing of it until the deaths rose to four and five a day when he reported the matter to an officer of the Division. From the material submitted by the farmer it was evident that he was losing stock from paramphistomiasis and the farm was visited and several autopsies held. Local inquiries confirmed my suspicions that farmers must occasionally lose heavily from this malady. One old farmer assured me that vleis (marshy grounds) are apt to kill sheep in the beginning of winter, especially if there had been plenty of rain during the summer. As oesophagostomiasis is not severe, and liver flukes are unknown in that area, it must be assumed that these farmers have in the past lost sheep from paramphistomiasis.

In years gone by the pathogenicity of the paramphistomes has evidently been overshadowed by the presence of other helminths or poisonous plants. If Mr. Hobbs' sheep had been running on *Cucumis* sp. infested lands, this plant would undoubtedly have been pronounced the lethal agent. I may perhaps mention that Mr. Hobbs had one flock of sheep on a land with *Cucumis* abundant, and yet he has not lost a sheep there.

Most veterinary parasitology textbooks consider paramphistomes as non-injurious parasites. Some, however, believe that they may be harmful while others add that paramphistomes have occasionally been suspected of being harmful. Wooldridge mentions that these trematodes have been reported as pathogenic from Australia. In South Africa the Veterinary Division has always considered them as of little importance and farmers have been informed that they are not very harmful. In the situation (rumen) in which they are usually observed, they are undoubtedly not very injurious if at all. Immature paramphistomes from the duodenum of cattle and sheep have been reported from different parts of South Africa and from India. The Onderstepoort helminthological collection contains specimens collected from different parts of the Union. When in 1925 I recorded their presence in trek oxen dying on the Empangeni Townlands, the then Director of Veterinary Education and Research, Onderstepoort, informed me that he had had specimens from various parts of the country. There are specimens here collected from sheep and presented by Mr. Simson (1926), Government Veterinary Officer, Queenstown. Mr. Henderson, Government Veterinary Officer, Kokstad has last year and this year submitted specimens from sheep in his area.

The material from cattle consists of specimens submitted by the Principal Veterinary Officer, Basutoland, from cattle at Maseru, and specimens collected from cattle at Onderstepoort and at Empangeni, Zululand. In cattle, as in sheep, there is a diarrhoea, which is more profuse in bovines and has to be differentiated from Johne's disease and certain other forms of parasitic diarrhoea.

In an article headed "Some Problems in Sheep Diseases," Baldrey (1906), from India, discusses "Gillar" and points out that clinically it somewhat resembles fascioliasis. He describes the pathological lesions and states: "The really pathogenic lesions will be seen in the small and large intestine, noticeably so in the pyloric region of the former. Here there will be seen an enteritis, the mucous membranes being necrotic and probably shredding off in places . . . The veins in these areas will have quite a varicose appearance." He further records that he collected amphistomes "in an undeveloped state" from the haemorrhagic area. Specimens were submitted to Looss, at Cairo, who identified them as immature amphistomes. Although Baldrey writes: "In subsequent post-mortems the presence of immature amphistomes was found to be constant and always in the diseases duodenum and pylorus," he did not suspect these parasites as the causal agent because ". . . it is supposed that these organisms have not a very serious effect, ..." This interpretation of Baldrey recalls Cobbold's warning ". . . the student of helminothology must, as a primary discipline, dispose himself of all preconceived opinions whatsoever . . ."

Walker (1906), in a preliminary note discusses this ovine disease "Gillar." According to him, it is practically confined to low-lying swampy areas along the banks of rivers and in parts which are often flooded during the rainy season. The malady is as a rule at its worst during the months of December, January and February. That season would correspond to our winter. He is of the opinion "that the

parasite to which it is due passes some period of its existence in marshy places, and that it gains access to the host with the food in September and October, i.e. when the rains and flooded pastures are drying up."

Cotylophoron indicum, Stiles and Goldberger, 1910, has been recorded from *Ovis aries* in India and is according to Maplestone (1923), synonymous with *Cotylophoron cotylophorum*. Fischoeder described the species originally from the stomach (presumably the rumen) of *Bos taurus*, Togo and *Bos zebri*, German East Africa. Maplestone (1923), records it from the stomach of bullocks in Sierra Leone; buffalo and nswala in the Upper Shire River district, Nyasaland; Pagan dwarf bull from Ilorin, Northern Nigeria; waterbuck from Zeref, Khartoum; hartebeest, Nyasaland; antelope sp., Rhodesia and Nyasaland. Ortlepp records it from the rumen of sheep in the Vryheid district, Natal. Mr. D. T. Mitchell, M.R.C.V.S., presented me with specimens from the rumen of a goat slaughtered at the Rangoon Abattoir. Cobbold (1875), records *Amphistomum tubercutatum* from the intestine of Indian cattle. This species may be identical with *C. cotylophorum* whose immature stages have slightly developed cuticular tubercles on the anterior portions of the body. Curson (1928), records *Cotylophoron cotylophorum* as a parasite of the African buffalo in Zululand. At Onderstepoort I have recovered it from both cattle and sheep. From these records it is evident that *C. cotylophorum* has a wide distribution.

The Symptoms Manifested by Infected Sheep.

The symptoms are by no means characteristic for this disease only. The animal appears dull and listless, feeds badly, even when turned on to green oats, and tires readily when driven. The general symptoms of unthriftiness increase rapidly as the disease progresses, the wool becomes dry and harsh, and is easily pulled out. The visible mucous membranes are pale. Oedema of the sub-maxillary subcutaneous tissues develops and as with fascioliasis and other anaemic disorders, it is most pronounced in the evening, while it has practically disappeared by the next morning. A foetid diarrhoea develops early and continues until the animal dies. In a case which showed signs of recovery the autopsy revealed that the parasites had migrated to the rumen.

Post-mortem Appearances.

Seven animals have been destroyed for autopsy. The blood was rather anaemic and stained the fingers poorly. The subcutis contained a fair accumulation of fat showing disseminated fat necrosis. This fat necrosis was general, but was most marked in the intra-abdominal and intra-thoracic collections of fat. In the more chronic cases there was well marked hydro-pericardium, hydro-thorax, hydro-peritoneum, and oedema of the mediastinal, mesenteric and sub-maxillary tissues. Most of the livers showed slight fatty changes. The most pronounced pathological changes were manifested by the first four to eight feet of the duodenum. The blood vessels on the peritoneal surface were engorged and tortuous imparting a cyanosed appearance to the gut. On section the duodenal wall was thickened, the mucosa particularly so. On the congested mucous surface numerous small, flesh coloured

objects were present. These were in some cases also recovered from the less congested posterior portion of the gut, as far as the anus. In older standing cases, sand, small pebbles and other foreign bodies half filled the abomasum. In still older cases observed at Onderstepoort some parasites were recovered from the rumen.

The Life-cycle of the Parasite.

Before prophylactic measures can be successfully and scientifically instituted against this disease, it is essential that the time required for the completion of the different stages in the development of the causal parasite be known.

Snails collected from the pool on the farm "Breezy Brae" and live snails sent by Mr. Hobbs, "Haldon, Petrus Steyn," proved on examination to be infected with an amphistome cercaria, practically identical with *Cercaria frondosa* as described by Faust and claimed by Cawston to be the larval stage of *Paramphistomum calicophorum*, Fischoeder, 1901, which is considered by Maplestone (1923), as identical with *Paramphistomum explanatum* (Creplin, 1847), Fischoeder, 1901.

The developmental stages met with in the livers of dissected snails were sporocysts, rediae, daughter rediae and cercariae. The cercariae when kept in water in a glass dish encysted firmly on the sides of the vessel near the surface of the water or on blades of grass suspended in this water. The encysted cercariae are dark brown in colour and can just be seen with the naked eye. When they were present in a Petri dish containing a shallow layer of water they encysted at the bottom but were never seen floating about in the encysted stage. They cast their tails before encysting. If the encysted trematode is now ingested, it arrives in the duodenum where it attaches itself, feeds and grows until it migrates to the rumen to mature and oviposit. The contents of the duodenum being rich in semi-digested material is undoubtedly most suitable food for the young paramphistomes. This selection of the duodenum by the young forms and their ultimate migration to the rumen illustrates Cobbold's lines: "None of the internal parasites continue in one stay; all have a tendency to roam; migration is the soul of their prosperity; . . . . To attack, to invade, to infest, is their legitimate prerogative. Their organization, habits and economy are expressly fashioned to this end."

On its migration to the rumen its life as a *true* parasite would appear to cease. There is no evidence that the host is injured to any appreciable degree by its sojourn in the rumen. Van Beneden (1889), discussing "Free messmates" writes: "Worms which have less freedom, like the Distomians, are sometimes both messmates and parasites."

C. cotylophorum is a true parasite while inhabiting the lumen of the duodenum, but would seem to inhabit the rumen for protection. The observations on the migration of the species under discussion confirms Looss' observations that amphistomes first inhabit the small intestine before "they return to the first stomach which is their definite habitat."

The Rate of Development in the Vertebrate and Mollusc Hosts.

From the available data it would seem as if the development of the parasite in the verebrate host is very slow, and that approximately six to eight weeks are spent in the duodenum, while another eight weeks will probably elapse before eggs are passed. At present there is no indication as to the time required for the completion of the cycle in the mollusc. The two outbreaks investigated suggest that the snails become infected in the summer and that encysted cercariae are abundantly present in the vegetation by about May. That some may survive the winter in the snail and emerge early in the summer is likely, but undoubtedly not of common occurrence. It is doubtful whether infected snails would survive the severe winters experienced in those parts.

On the farm " Haldon " the history of the outbreak suggests that infected snails had either survived the winter of 1928 and that the cercariae liberated the following summer infected the sheep in the autumn. The infected camp carried cattle since December, 1928, and it seems probable that these cattle must be held responsible for the infection of the snails which transmitted the parasite to the sheep introduced in May. According to the owner the sheep showed symptoms a fortnight to three weeks after entering this particular field. That the cattle running in the infected camps at " Breezy Brae " did not appear affected, may perhaps be explained by the fact that cattle do not graze as close as sheep. If infection was caused by the water ingested, one would have expected the cattle to have shown symptoms such as diarrhoea.

Treatment.

Tests with carbon tetrachloride proved that this drug had beneficial effects only when administered in doses of from 8 to 10 c.c. in raw linseed oil. The simultaneous administration of magnesium sulphate or calcium lactate should, according to various workers, reduce the toxicity of the drug. Infected animals should receive bonemeal to compensate for the loss of calcium. The giving of lime to reduce any hyperacidity of the abomasum may be advisable and is worthy of a trial. There is plenty of scope here for the biochemist, and those interested in pathological physiology.

Prophylactic Measures.

Sheep and freshwater snails are usually never successfully reared in the same field. Snail-infested vleis should, where practical, be drained or fenced off. Local conditions should be studied before advising the farmer which measures to adopt. Mr. Hobbs suggested draining the vlei and planting willows to aid still further in the reduction of the moisture present.

The destruction of the snails by chemicals such as lime or copper sulphate, may be tried. The latter may give good results where the water is not too muddy or too heavily polluted with organic matter. The use of bonemeal and salt lick should be advocated in all infested areas.

The freshwater snails met with in the Union, are imperfectly known, and I appeal to all veterinarians to study their habits, and to submit live specimens for determination, by animal experimentation, of the cercariae present and likely to infest domestic stock. Live snails packed in moist vegetation travel well by post or by passenger train.

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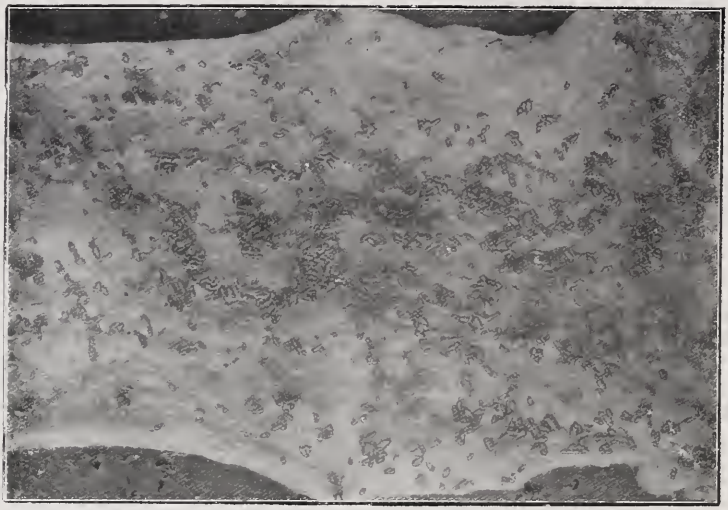


FIG. 1.

Fig 1. Photograph of a portion of the duodenum showing mass infestation with immature paramphistomes. Natural case from "Breezy Brae."

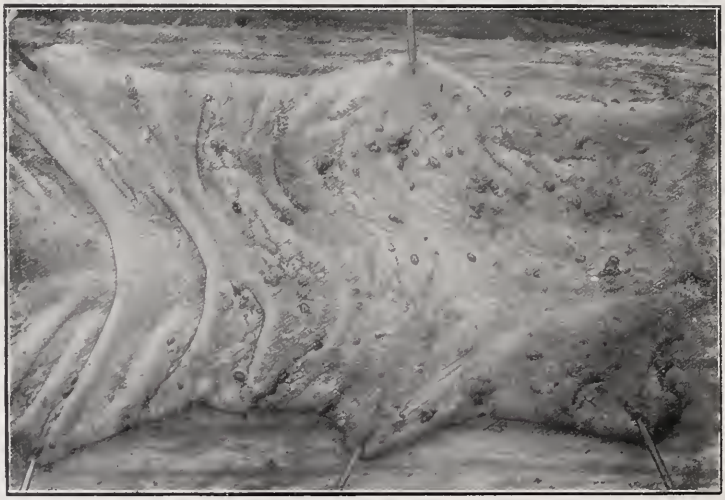


FIG. 2.

Fig. 2. Photograph of a portion of the duodenum showing verrucose appearance of the mucosa and whitish raised areas (sites of attachment). Another natural case from "Breezy Brae."

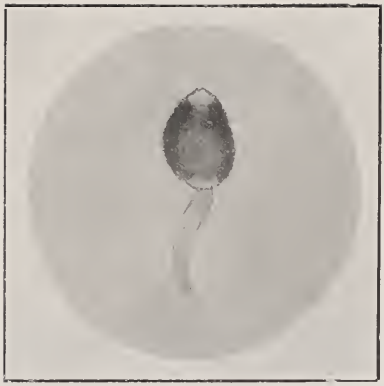


Fig. 3.

Fig. 3. Microphotograph of a cercaria showing "eye"-spots. Specimen from the liver of *Bulinus* sp. off "Haldon."

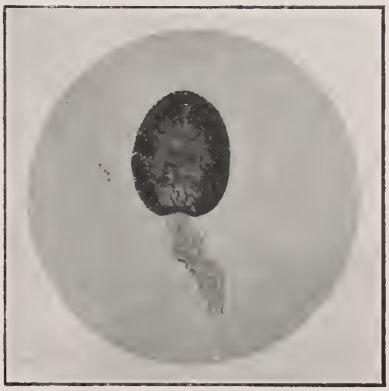


Fig. 4.

Fig. 4. Microphotograph of a cercaria after escape from a snail off "Haldon." Note pigment distributed throughout the body.

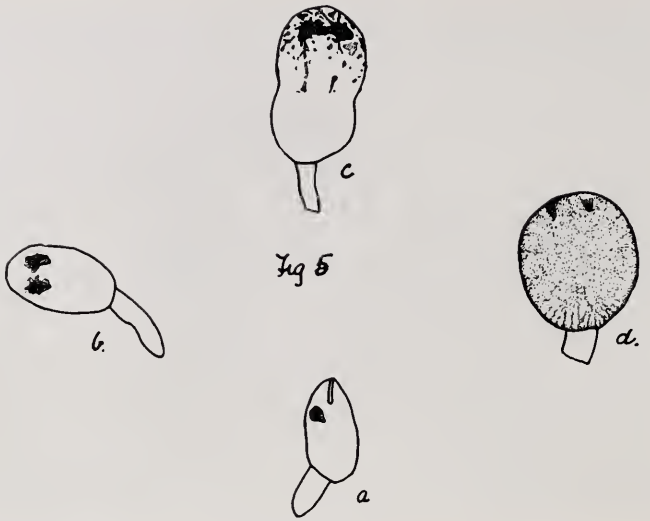


Fig. 5. Cercariae from a snail off "Haldon."

a. Lateral view of an immature cercaria showing one "eye"-spot.
b. Dorsal view of an immature cercaria showing the two "eye"-spots.
c. A dorsal view of a more matured cercaria showing radiation of the pigment from the "eye"-spots.
d. Ventral view of an almost matured cercaria showing the "eye"-spots and the pigment disseminated throughout the body.

FIG. 6.

Fig. 6. Photograph of a plant with *Bulinus* sp. adhering. Plant removed from the snail infested pool at "Breezy Brae."

Fig. 7.

Fig. 7. Portion of Rumen showing *Cotylophoron cotylophorum* adhering.

Tsetse-Fly Survey of Zululand and Surrounding Territories.

By J. L. PAPERT, B.Sc., Research Officer.

In June, 1928, I received instructions from the Director of Veterinary Services to proceed to Zululand on a tsetse-fly survey, the main object of which was to delineate the eastern limits of the fly. My staff consisted of six natives who had spent long periods on tsetse-fly work during previous investigations in Zululand and so were familiar both with the tsetse and its haunts. I later increased the number of native assistants to twelve. Both donkeys and cattle were used as bait animals and the procedure adopted was to tether these animals in suspicious localities where the boys caught flies from them. As a preliminary step I toured Zululand in order to become familiar with the country and so be able to draw up my plans of investigation.

Description of Country.

The following is a very rough description of the bush conditions existing along the eastern side of Zululand.

In the Lower Umfolozi Division the bush does not extend further east than the Ntambanana Settlement. There is, however, a large exotic plantation about three miles east of Empangeni village which has to be taken into consideration as nagana has been diagnosed on the farms bordering this plantation. The Umfolozi cane farms are open farms, but between them and the mouth of the Umfolozi River there is a large stretch of dense bush about 47,000 acres in extent. The valley of the Umfolozi River running through these cane farms is devoid of bush.

Between the Inyalazi River and Lake St. Lucia there is scattered bush which becomes denser on moving northwards until in the peninsula between False Bay and Lake St Lucia it is very dense.

The Hluhluwe River valley is very densely bushed. The southern portion of the Hluhluwe Settlement, with the exception of the farms bordering the river, forms an open plain. The northern portion of the settlement is covered with scattered bush. Between the settlement and the west bank of False Bay there is very dense bush. This bush mass continues to the south to Entontweni and then connects up with the Hluhluwe Game Reserve. To the north it extends to the valley of the Mnnywana which forms a wide break in the bush. To the north of the Mnnywana the bush mass continues to the Umkuzi River and includes the north-western portion of Native Reserve No. 1.

Shooting area No. 7, lying between the Hluhluwe farms and the Hluhluwe Game Reserve is covered with sparse bush. Native Reserve No. 2 is mainly open country with bushy kloofs and patches of bush here and there. Native Reserve No. 13 is bushed and continuous with the Umkuzi Game Reserve.

North of the Umkuzi River there is lalla palm country to the east of the Mozi River and bushed country to the west. The Mozi River would be roughly the eastern border of the bush which extends from the Umkuzi Game Reserve to the Portuguese border. This bush is bounded on the west by the Lebombo Mountains. The palm country extends between the Mozi and Mbazwane Rivers to the Maputa River and is about 16 miles wide. In this belt are small bush masses the most important of which is the Manywane bush lying around Mseleni, where clinical cases of nagana were encountered in June, 1928. There is also a narrow peninsula of heavy bush at Nibele along the eastern boundary of Native Reserve No. 1. Nagana is said to have been prevalent in this area about ten years ago.

Empangeni Rail.

During July, 1927, it was definitely ascertained that nagana was the cause of the large number of deaths amongst cattle on the cane farms lying to the east of Empangeni village. This was not the first occurrence of the disease on these farms but in previous years nagana was never suspected as these farms are situated on open country about 30 miles from the Umfolozi Game Reserve and tsetse have always been associated with game and bush. In August, 1927, I was sent to study conditions prevailing in this area in order to arrive at some conclusion to account for this outbreak of nagana. In reviewing the history of the animals infected I found that none of them had been introduced from nagana areas within the few years previous to the outbreak and hence concluded that they must have become infected on their respective farms. This, of course, presupposed the presence of fly on the farms. Fly could have been introduced into this area either (1) accidentally, by being carried there in motor vehicles travelling from fly belts, or (2) naturally, by the fly moving along bushed valleys during the course of its summer extensions.

Lying in between the infected farms and in close proximity to Enpangeni Railway Station is a large plantation belonging to the Forestry Department. As there is a continuous stream of motor traffic between Ntambanana Settlement and the station it is quite possible that fly was accidentally carried to the station. Such fly may then have become marooned in the fringes of the plantation. As the animals living in the heart of the plantation were not suffering from nagana it would appear that the fly did not penetrate too deeply into the plantation. Baiting along the fringes of the plantation did not, however, reveal any tsetse.

Along the eastern boundry of these farms and flowing from opposite directions into Lake Insese are the Enseleni and the Umhlatuzi Rivers. Both these rivers flow through tsetse country and thus it is likely that fly move down their valleys during summer. Tsetse have been found on the Umhlatuzi River just north of Native Reserve No. 7B which adjoins these farms. This would therefore be the most likely theory to account for nagana in this area.

It may be of interest to add that the vicinity of the plantation is badly infested with the blood-sucking fly., *Stomoxys calcitrans*, whose abundance is no doubt due to the special breeding ground supplied by the sugar-cane mill furrow present there.

Ntambanana Settlement.

During the summer of 1927-1928 professional hunters had been engaged in destroying the large herds of zebra and incidentally any other game that were found in the buffer area lying between Ntambanana Settlement and the Umfolozi Game Reserve. It was believed that through its wandering habits the zebra was a means of carrying the fly from the reserve to the settlement and with its destruction the incidence of the disease would be greatly lessened in the settlement.

While visiting this buffer area in June, 1928, I found that it was practically barren of animals for no game were to be seen, but not devoid of tsetse for on the 15th of June two specimens of *Glossina pallidipes* alighted in my motor-car when about midway between the settlement and the reserve.

At this time the settlement was suffering from nagana to a greater extent than for previous years.

Dukuduku Bush.

From time to time cattle have died from nagana on the banks of the Umfolozi River in the Matubatuba district. It was then presumed that the bush lying to the north of the river and known as the Dukuduku bush was infested with tsetse.

The Umfolozi cane farms are situated on the coastal sand flats and are on the whole not bushed. They are situated both on the south and the north sides of the river.

The Dukuduku bush presents a more or less well-defined margin to the west and north, being bounded on the west by cane farms and on the north by open sand flats dotted with palm trees. To the east and south the bush merges into reed-covered swamps which extend to Lake St. Lucia and the Umfolozi River.

The Umfolozi valley is free from bush for at least eight miles in the direction of the Umfolozi Game Reserve. Thus the Dukuduku bush appears to be an isolated dense mass of bush not connected with the nearest definitely known fly area, namely the Umfolozi Game Reserve.

The bush is about 47,000 acres in extent, and is divided into two main portions by a large glade running north to south. The portion lying to the west of the glade is very dense, particularly so towards the Umfolozi River and Umfulululi Lake in the South. The portion to the east is broken up into smaller masses with open spaces in between. These open areas are occupied by natives who keep large herds of cattle. Duiker, bushbuck and reedbuck are still to be found in the bush, and in the swamps are hippopotami and crocodiles. Bird life is plentiful.

Twenty-three days, from 28th June to 23rd July, were spent in baiting the bush, but no tsetse were encountered.

The cattle living in the bush were sleek and fat in appearance and had never, according to evidence obtained in the district, been ravaged by nagana.

It is difficult to explain the cases of nagana that have occurred in the territory neighbouring the bush unless we assume that the disease had been contracted elsewhere.

Hluhluwe District.

The Hluhluwe and Umkuzi Settlements are continuous and occupy roughly a Y-shaped portion of the country. They are bounded on the west by Native Reserve No. 12 with the Hluhluwe Game Reserve lying towards the south-west portion. To the south the Hluhluwe River marks the boundary and to the east the settlement is bounded by the heavy coastal bush. The two arms of the Y rest on the Mkuzi River to the north with Native Reserve No. 2 to the left and the Umkuzi Game Reserve to the right occupying the hollow between the arms. The Lebombo Mountains effectively separate the Umkuzi Settlement from the Umkuzi Game Reserve. The Hluhluwe Settlement is, however, in close proximity to two game reserves, namely the Hluhluwe Game Reserve to the south-west, and the Umkuzi Game Reserve to the north.

The present northern boundary of the Hluhluwe farms is the Munywana River. The land to the north of this has, however, been surveyed into farms and applications for some of these had been made by existing settlers. In one instance the farms lying in the extreme north-eastern portion of the settlement, bordering the Umkuzi River, had been occupied by the end of the winter of 1928.

Southern Portion of Hluhluwe Settlement.

The southen portion of the Hluhluwe Settlement does not on the whole present the type of country suitable for tsetse. The central farms are situated on an open plain, whereas the peripheral farms are either bushed or in contact with bush. In July, 1928, only those farms bordering the Hluhluwe River and the special shooting area which separates the settlement from the game reserve were infected with nagana. The central farms and those to the east were free. The farms situated in the south-western corner of the settlement had been early abandoned owing to the ravages of the fly.

It was very surprising to find that the farms along the eastern side of the settlement were not suffering from nagana as these farms are encroached upon by dense bush and in one instance the farm is actually situated in the heart of the bush. This bush extends along the west bank of False Bay and the mouth of the Hluhluwe River. Duiker, bushbuck, reedbuck, inyala, waterbuck, hippopotami and crocodiles are fairly plentiful. Conditions here are therefore very favourable for the fly, but during a period of 16 days, from the 27th of July to the 11th of August, spent in baiting the bush no flies were encountered.

The bush is well populated by natives who keep large herds of cattle, and these animals were in a very fit condition at the time when those on the Hluhluwe farms to the west were dying from nagana. The disease was absent also from the farm (Mr. Mason's), which is situated in the bush.

From evidence obtained from both Europeans and natives it is certain that natives have kept stock in this bush for many years without experiencing nagana.

Nagana is present on the farms situate in the south-eastern corner of the settlement, but as no fly was found there we must conclude that the valley of the Hluhluwe constitutes a summer extension of the fly. Thus we have the fly on the outskirts of the coastal bush during certain periods of the year and as it does not penetrate this bush it would appear that the littoral bush is in some way unfavourable to the fly.

On the 28th of July, 1928, a specimen of *Glossina brevipalpis* was caught on the back of a native while crossing a bushed donga in shooting area No. 7, which lies between the settlement and the reserve. On the evening of the 4th of August, two specimens of *G. pallidipes* were found in this area. This area would therefore constitute the eastern limits of the fly in the southern portion of the Hluhluwe Settlement, with, however, summer extensions along the Hluhluwe River valley and other bushed valleys extending into the settlement.

Northern Portion of the Hluhluwe Settlement.

The northern portion of the settlement extends at present to the Munywana River in the north, but it is gradually being extended beyond this. It presents typical fly country. Game is still present and occasionally a herd of wildebeest is seen.

The Munywana River, like most of the Zululand rivers flows only during the rainy season. During the winter of 1928 it presented a dry bed with a few pools here and there. The two main pools were situated on farm Golweni and were used by all the neighbouring farmers to water their stock. As large numbers of these animals were suffering from nagana it was decided to bait the bush surrounding these pools.

During the period 14th to 23rd of August a specimen of *G. pallidipes* was caught on a bait animal.

The Munywana flows into the Msinene River, which is also a dry stream in the winter. From the 28th of August to the 22nd of September pools in the Msinene bed were baited. A single specimen of *G. pallidipes* was caught on the 12th.

The whole of this portion of the settlement would, therefore, constitute a fly belt which extends eastwards to Native Reserve No. 1.

Lower Umkuzi Drift.

This drift, where the old coast road from Hluhluwe Railway Station to the northernmost parts of Zululand crosses the Umkuzi River, has always been looked upon as a bad fly haunt. It is situated in close proximity to the eastern corner of the Umkuzi Game Reserve. Herds of wildebeest are commonly met with in its vicinity.

A period of 24 days, from the 25th of September to the 18th of October, was spent in baiting the bush surrounding the drift and extending to the junction of the Umsindusi and Umkuzi Rivers, without, however, encountering testse.

Native cattle are common in the area between the Munywana River and the Umsindusi River and occur as far north as the drift. There is also a continuous stream of donkey wagons on the old coast road between the drift and the station. At the time of the survey nagana was not known among these animals. It is to be noted that the road crosses both the Msinene and the Munywana Rivers and hence passes through a definite fly belt.

Ox wagons are used for transport purposes north of the drift. At Manguzi, animals used for this purpose were found to be suffering from nagana, and as no other animals there showed symptoms of the disease, it is evident that the disease was contracted either at the drift or at some point along the road.

It was then decided to postpone further investigations in this area until some future date and devote the summer months to making observations in the Ngotshe district.

Ngotshe District.

The tract of country west of the Lebombo Mountains and lying between the Umkuzi River and the Pongola River forms the eastern portion of the Ngotshe district of Natal. In bush conditions it is similar to the country lying to the south of the Umkuzi and is suitable for fly. This area has been occupied only within recent years by farmers interested in cotton planting. With this occupation the large population of game previously found in this district was greatly depleted. Natives have always kept cattle in the vicinity of Pongola Poort and in the years following the Boer War it had been the custom of the Vryheid and other highveld farmers to trek their cattle to this area for winter grazing.

From 8th November to 5th December the survey party was concentrated on farm Leeuwkop, south of the Pongola, and situated on the Rooirand Hills. Clinical cases of nagana were seen but no *Glossina* were encountered. It was then decided to divide the party, one portion to have a base at Wanhoop and work westwards along the Pongola River, and the other to work westwards along the Umkuzi River from Mooiplaats. Fly was fairly plentiful on Mooiplaats and the following tabular statement shows the result of the investigations in the district.

Farm.	No. of *G. Pallidipes* Caught.	Date.
Mooiplaats Doornhoek	13	December, 1928
Welcome	1	23/2/29
Bedrogen Onrecht	1	27/2/29
Bethel	1	28/2/29
Pongola	2	9/4/29
Leeuwspoor	1	10/4/29
Wanhoop	2	13/4/29

It was thus found that fly was not only present along the two rivers, the Umkuzi and the Pongola, but also in the intermediate country, namely at Bedrogen Onrecht and Bethel. As the main road from Candover Railway Station westwards runs through these two farms it is quite possible for the infection to be carried further inland.

A further point of interest attaches to the find of *Glossina* on Welcome. This farm is situated west of the Rooirand on the north bank of the Umkuzi. For the past 25 years it has been used as a stock farm and during that period the owner had not experienced nagana. The fly must, therefore, have encroached so far west during recent years.

No *Glossina* were encountered west of Welcome but as the country here is still suitable for the fly it is quite possible for the fly-belt to be extended throughout the whole of the low-lying portion of Ngotshe.

Piet Retief District.

To the north of Ngotshe and lying between the Pongola River and the southern boundary of Swaziland is a narrow strip of country belonging to the Piet Retief District. As this is also typical fly country and as the fly had been found on the south bank of the Pongola baiting was commenced in the bush to the north of the river.

This strip of country had been allotted to settlers at the end of 1928, but by June, 1929, only three settlers had occupied their farms and introduced cattle into the district. This district had been opened up with the extension of the north coast railway line across the Pongola.

In between the farms there is a mass of dense bush, known as the Gollel Bush, which has been reserved for game. On the 10th of June a specimne of *G. pallidipes* was caught in this bush. The theory that the fly had been distributed north by the train service is most unlikely, for, according both to European and native reports, nagana has been prevalent in this vicinity for many years.

This is the only record of the capture of *Glossina pallidipes* in the Transvaal.

Swaziland.

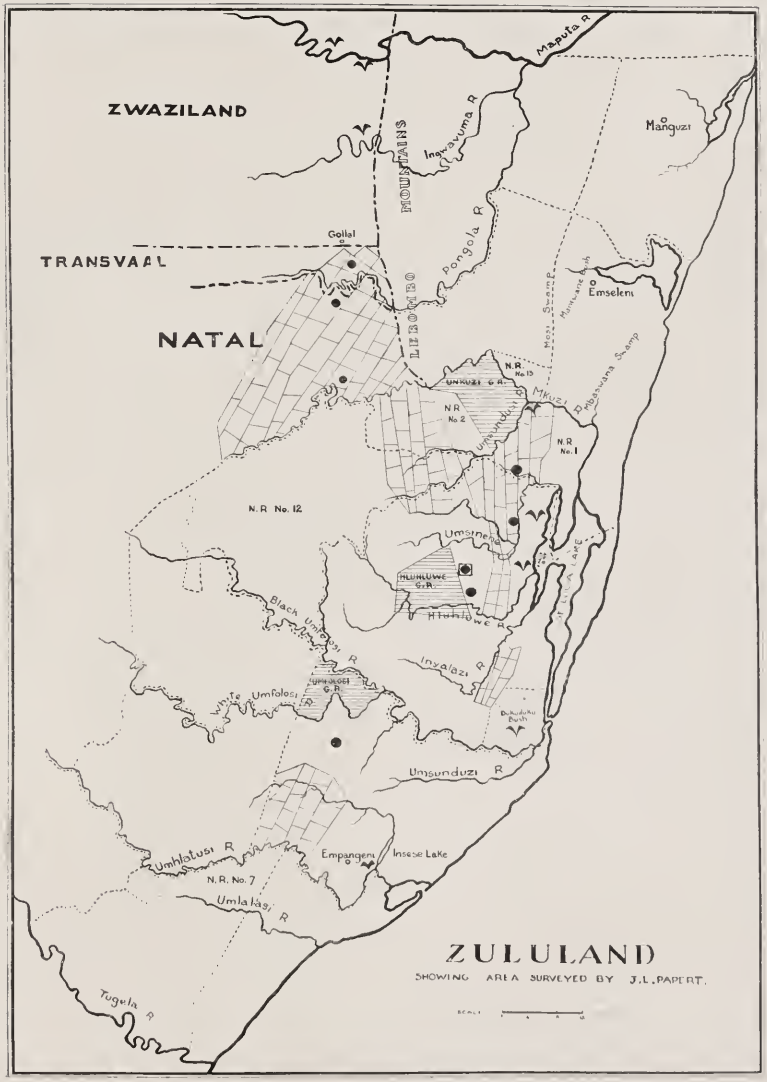
The eastern low-lying portion of Swaziland is topographically a continuation of the Ngotshe district and is thus suitable country for the fly.

From the 24th of June until the end of the investigation, the 25th of November, the survey party remained in Swaziland baiting the country between the Lugwavuma River and the Usutu River, without, however, encountering tsetse.

It would appear from available evidence that *Glossina* at one time extended in a northerly direction through Swaziland and linked up with the fly areas known to have existed a generation ago in the Barberton district. It should be noted, however, that the fly caught at Barberton (Louw's Creek) was *G. morsitans*, but how far south this species extended and how far north *G. pallidipes* still ranges is uncertain.

SWAZILAND
TRANSVAAL
PIET RETIEF
NATAL
NGOTSHE
ZULULAND
LEBOMBO RANGE
PONGOLA RIVER
Middelin
Lebumbo Poort
Ophuruni Store
Mhlumeni Store
Leeuwkraal
Nkravosi Heuvel
Wildebeest druai
Long Kloof
Geffel
Kroonrug
Koodoosberg
Doornhoek
Enfermis
Burgersdorp
Uitzicht
Oasis
Bedrog
Steenbok laagte
Prospect
Wilhelmina
Dordrecht
Doornhoek
Buchhoek
Zungwini
Ongeluk
Deurn
Spioenkop
Ulundi
Ubombo
GLOSSINA
NAGANA
SCALE
Miles

Map 1.


ZWAZILAND
TRANSVAAL
NATAL
LEBOMBO MOUNTAINS
Maputa R
Ingwavuma R
Pongola R
Golela
Manguzi
Emseleni
N.R. No. 13
UNKUZI G.R.
N.R. No. 2
N.R. No. 1
N.R. No. 12
Umsinene
HLUHLUWE G.R.
Hluhluwe R
Black Umfolozi R
White Umfolozi R
UMFOLOZI G.R.
Inyalazi R
Umsunduzi R
Umhlatuzi R
Empangeni
Insese Lake
N.R. No. 7
Umlalazi R
Tugela R
ZULULAND
SHOWING AREA SURVEYED BY J.L.PAPERT.
FLIES FOUND (G PALLIDIPES)
G. BREVIPALPIS
LOCALITY BAITED BUT NO FLY FOUND

Map 2.

Section V.

Mineral Deficiency and Metabolism.

P. J. du Toit and H. H. Green	Dicalcium Phosphate as a Source of Phosphorus in Nutrition.
A. I. Malan... ...	The Increase of Inorganic Phosphorus due to Hydrolysis in Solutions of Blood.
J. G. Bekker and S. D. Rossouw	A Note on Some Conditions in Sheep in the Strandveld of the Bredasdorp District.
S. D. Rossouw...	Studies in Mineral Metabolism X. The Effect of Bone-meal Feeding on the Phosphorus Partition of the Blood of Sheep.
A. I. Malan... ...	Studies in Metabolism XI. Mineral Metabolism and Blood Analysis.
P. J. du Toit, A. I. Malan, and S. D. Rossouw	Studies in Mineral Metabolism XII. Phosphorus in the Sheep Industry. Preliminary Report.
A. I. Malan... ...	Studies in Mineral Metabolism XIII. The Phosphorus Partition of Blood of Some Animals.
J. S. Otto	Micro-Method for the Determination of Antimony in Organic Material, and of its course through the Animal Body.

Dicalcium Phosphate as a Source of Phosphorus in Nutrition.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services; and

H. H. GREEN, D.Sc., late Sub-Director of Veterinary Services.

Long before the relation between lamsiekte and phosphorus deficiency was understood it was known that the feeding of bonemeal to cattle had a marked influence in removing "pica" and reducing the incidence of the disease. When, in 1919, the etiology of lamsiekte was cleared up, the explanation of this beneficial action of bonemeal became simple. It was then shown that lamsiekte was ultimately due to a phosphorus deficiency in the soil and pasture and, as soon as this deficient mineral was supplied in the form of bonemeal, the disease disappeared.

In the course of these investigations it was also found that bonemeal, when fed to cattle in the phosphorus deficient areas of South Africa, had a marked effect in stimulating growth, increasing fertiltiy, producing more milk, reducing mortality, and improving the animals generally. This phase of the phosphorus problem has been discussed in detail in recent publications.

In the present article it is intended to deal with one aspect of the problem only, namely, the substitution of some other phosphorus compound for bonemeal.

EARLIER EXPERIMENTS WITH VARIOUS PHOSPHORUS COMPOUNDS.

In the detailed report on lamsiekte published by Theiler and his co-workers in 1927, experiments are recorded which were carried out in 1919-1920.

Sodium phosphate was given to cattle and rapidly reduced the osteophagia. A daily dose of 3 oz. per head was not quite sufficient, but 6 oz. per head per day removed the osteophagia completely.

Pure precipitated *calcium phosphate* was given in amounts varying between 2 oz. and 5 oz. per head per day with entirely satisfactory results; 4 oz. was sufficient to remove the osteophagia altogether.

Phosphoric acid gave similar results. The acid was given either in the drinking water or in the form of a maize paste. Quantities of 20 to 25 c.c. acid (equivalent to 15 to 18 grams phosphoric oxide) were required to remove the osteophagia.

Experiments were also carried out with finely ground *rock phosphate* which was found to have little effect when given in quantities of 4 oz. per head per day; also with "*Saldanha Bay phosphate*" in its crude state (which proved to be ineffective) and after conversion to its "citric soluble" form (in which form a dose of 5 oz. per head per day gave good results).

Wheaten bran, which is comparatively rich in phosphorus, was also used with satisfactory results; 2 lb. per head per day was found to be enough to remove osteophagia.

In all these experiments the sole object was to test the efficacy of these substances to remove osteophagia. Weight records of the animals were not kept, nor was the influence of these substances on milk production or other functions of the animals studied.

CALCIUM PHOSPHATE AS A SUBSTITUTE FOR BONEMEAL.

In 1924 Theiler, Green and Du Toit expressed the opinion that the "best and cheapest form" of phosphorus was *bonemeal*, which had "the added advantage of being palatable, of satisfying the specific craving, and of offering least trouble in administration." In regard to "*precipitated calcium phosphate*" they say that it "behaves very like bonemeal, but is more expensive, tasteless and more troublesome to administer; especially since the cattle do not recognize it and do not take it of their own accord."

In spite of the opinion held by these authors, it was decided in 1927 to carry out a further test with calcium phosphate in order to get an accurate comparison between this compound and bonemeal as a source of phosphorus in the nutrition of cattle on phosphorus deficient veld.

The question of *palatability* was not considered to be of great importance, since both bonemeal and calcium phosphate were *administered by means of a spoon*, as described previously. This practice has been adhered to throughout the experiments which were conducted at Armoedsvlakte during the last 10 or 11 years; it has also been followed very largely by farmers all over the country. In spite of its obvious disadvantages this method of administering bonemeal has remained in favour because (1) it is *economical*, there being no wastage of material; and (2) it ensures that every animal gets the *correct dose*. If bonemeal is given as a lick in troughs the greedy animals will eat far more than they require and thus waste the material, whereas other animals will get less than is necessary for the removal of osteophagia and such animals are then liable to contract lamsiekte.

The method of dosing bonemeal (or other phosphorus compounds) by means of a spoon is not considered ideal—and attempts are now being made to find more practical methods—but in the study of the relative values of bonemeal and calcium phosphate it proved very satisfactory, inasmuch as it made an accurate comparison possible.

Of more importance in this experiment was the question of *cost*. Theiler, Green and Du Toit (1924) stated that bonemeal was the best and cheapest form of phosphorus. In comparison with calcium

phosphate bonemeal is undoubtedly cheaper weight for weight, but the question to be decided was: what is the *effective dose* of calcium phosphate and how does the *price of this dose* compare with the price of the effective dose of bonemeal. A reply to this question will be found at the end of this paper.

The dicalcium phosphate, also called precipitated calcium phosphate, used in this experiment was imported by a local firm of manufacturing chemists. It was prepared in the usual way by treating bones with acid, decanting or filtering the resulting solution of phosphoric acid from the insoluble residue and treating the former with milk of lime to precipitate the dicalcium phosphate. This product contains 42.6 per cent. P_2O_5, of which 39.5 per cent. is citric soluble.

THE EXPERIMENT.

The main object of the experiment was to compare calcium phosphate with bonemeal as regards (*a*) its ability to prevent pica, (*b*) its stimulating action on the growth of cattle, (*c*) its relative cost.

Arrangement of the experiment.—In order to get as accurate a comparison as possible between these products, 56 young oxen were selected and divided into 7 groups of 8 animals each. One of the groups (the controls) was subsequently increased to 14 animals, so that altogether 62 cattle were used in the experiment.

The experiment was started in October, 1927, when these animals were about 9 months old and had just been weaned. In selecting the animals an attempt was made to have the groups as uniform as possible, especially as regards weight.

The seven groups were treated as follows:—

All the animals were grazed on the farm Armoedsvlakte, in the Vryburg district, and received no supplementary food except the dose of bonemeal or calcium phosphate mentioned below, which, in accordance with the practice observed in all similar experiments at Armoedsvlakte, was given by means of a spoon to each individual animal every day except Sundays.

Group 1 (8 animals) received 3 ounces bonemeal daily.
Group 2 ,, ,, 1 ounce ,, ,,
Group 3 ,, ,, $\frac{1}{2}$,, ,, ,,
Group 4 ,, ,, $\frac{2}{3}$,, $CaHPO_4$,,
Group 5 ,, ,, $\frac{2}{3}$,, ,, ,,
Group 6 ,, ,, $\frac{1}{3}$,, ,, ,,
Group 7 ,, ,, no phosphorus.
Group 8 (6 animals) received no phosphorus.

In groups 1 and 4 the phosphorus ration was considered to be in excess of the requirements of young oxen such as those used in the experiment. These two groups were to be compared with each other.

In groups 2 and 5 the phosphorus ration was considered to be on the borderline of normal requirements.

The amount of phosphorus given in groups 3 and 6 was considered sufficient to effect improvement in growth (when compared with the controls), but not sufficient for optimum growth.

Groups 7 and 8 were intended to serve as controls for comparison with each of the other groups and to show the degree of improvement effected by the different doses of phosphorus.

The experiment was started on the 19th October, 1927, and lasted till the 2nd November, 1929, i.e. just over 2 years.

RESULTS OF EXPERIMENT.

For convenience each group will first be discussed separately and then the different groups will be compared with each other and conclusions drawn.

Group 1.

(*3 ounces bonemeal daily.*)

At the commencement of the experiment (19.10.1927) the *average weight* of the 8 animals was 441 lb. (highest 535, lowest 365 lb.). The animals increased rapidly in weight until May, 1928. During the ensuing winter months (May till October) there was a steady decline in weight. This loss of weight during the winter has always been observed in experiments at Armoedsvlakte and has been discussed in earlier publications. As soon as the spring vegetation appeared the animals again grew rapidly until June, 1929, when the influence of winter again began to be felt and growth stopped. From September until the close of the experiment in November, 1929, there was a further rapid increase in weight.

The *rate of growth* is illustrated by curve 1 in Figure 1.

The *average weight* of the 8 animals *at the conclusion* of the experiment was 1,032 lb. (highest 1,138, lowest 965 lb.), an increase of 134 per cent. over the initial average weight. Their condition was excellent.

In order to determine the value of a daily dose of 3 ounces bonemeal for the *prevention of pica*, all the animals in the experiment were tested once every fortnight in the manner described in other publications (compare Du Toit and Bisschop, 1929). The degree of pica in this group was low throughout the experiment. It varied from 0 per cent. to 50 per cent., but seldom rose above 25 per cent., i.e. only rarely did more than 2 of the 8 animals pick up a sterilized bone when it was offered to them.

Group 2.

(*1 ounce bonemeal daily.*)

The *average weight* of the 8 animals at the commencement of the experiment was 439 lb. (highest 518, lowest 363), i.e. practically the same as that of the animals in Group 1.

The *rate of growth* is indicated by curve 2 in Figure 1.

As in Group 1 the average weight increased until May, 1928, then dropped during the winter months, again increased during the spring and summer 1928-1929, and decreased once more during the winter 1929. There was a further rise from September to November, 1929, when the experiment terminated.

It will be noticed that the weight of the animals in Group 2 always remained lower than that of Group 1. Towards the end of the experiment the difference increased.

At the conclusion of the experiment the average weight of the 8 animals in Group 2 was 923 lb. (highest 1,056, lowest 834), an increase of 110.3 per cent. over the initial average weight.

In regard to the *prevention of pica*, the dose of bonemeal given to these animals proved to be much less effective than that given in Group 1. On many occasions 6 or 7 out of the 8 animals showed a craving for bones. On other days only 1 or 2 took bones; but, on the whole, the degree of craving was high, and towards the end of the experiment it remained high.

GROUP 3.

($\frac{1}{2}$ *ounce bonemeal daily.*)

At the beginning of the experiment the *average weight* of the 8 animals was 439 lb. (highest 496, lowest 330), i.e. exactly the same as the average weight of the animals in Group 2.

Curve 3 in Figure 1 shows how the weight of these animals increased. The same fluctuations are seen as were observed in the other two groups, namely, an increase in weight during the spring and summer months, and a decline during the winter months.

It will be noticed that whereas the weight curves of Groups 1, 2 and 3 started at the same point, curve No. 2 always remained below No. 1, and No. 3 always below No. 2. Just as in the case of curves 1 and 2, so it will be observed that the distance between curves 2 and 3 became greater as the experiment advanced.

At the conclusion of the experiment the average weight of the 7 remaining animals in Group 3 (one having died of lamsiekte on the 5th July, 1929) was 827 lb. (highest 992, lowest 720), an increase of 88.4 per cent. over the initial average weight.

The dose of bonemeal supplied to the animals in this group was quite ineffective in *preventing pica*. During the last 12 months of the experiment it was exceptional for an animal in this group *not* to chew a bone when it was offered one in the pica test. The fact that one of the animals *died of lamsiekte* towards the end of the experiment proves how ineffective the dose of bonemeal was to prevent pica and lamsiekte.

GROUP 4.

(2 *ounces* $CaHPO_4$ *daily.*)

The *average weight* at the commencement of the experiment was 439 lb. (highest 508, lowest 324), i.e. exactly the same as in the two previous groups.

The *rate of growth* is indicated by curve 4 in Figure 2. This curve shows the same general characteristics as were observed in the foregoing groups. It follows almost exactly the course of curve No. 1 (see Figure 3), keeping slightly below it during the latter half of the experiment.

At the *conclusion of the experiment* the average weight of the 8 animals was 1,000 lb. (highest 1,160, lowest 825), an increase of 127.8 per cent. over the initial average weight.

The *degree of pica* in this group was remarkably low. On many occasions none of the 8 animals took a bone, and on very many occasions only one animal (No. 2401) took a bone. This animal was apparently one of those " persistent cravers " which continue to chew bones simply as a " bad habit " in spite of an ample supply of phosphorus. Incidentally it may be mentioned that this same animal was also a very slow grower and was in a great part responsible for the lower average weight of this group compared with Group No. 1.

Group 5.

($\frac{2}{3}$ *ounce* $CaHPO_4$ *daily.*)

This group started with an *average weight* of 440 lb. (highest 515, lowest 317) i.e. practically the same as the average weight of the preceding groups.

Curve 5 in Figure 2 indicates the *rate of growth* of these animals. It will be noticed that this weight curve corresponds almost exactly with that of group 4, although the latter group received three times as much calcium phosphate as group 5. The significance of this result will be discussed later.

At the *conclusion of the experiment* the average weight of the 8 animals in Group 5 was 986 lb. (highest 1129, lowest 880), an increase of 124.1 per cent. over the initial average weight. Although this final weight is somewhat lower than that of Group 4, it will be observed in Figure 2 that the reverse was the case a month before the conclusion of the experiment. We may state, therefore, that these two groups behaved identically as regards increase in weight.

From the point of view of *degree of pica* Group 5 does not show up quite so well as Group 4 although it would appear that the dose of $\frac{2}{3}$ ounce calcium phosphate was sufficient to keep pica very low. On only three occasions throughout the two years did the " pica curve " rise above 25 per cent.

Group 6.

($\frac{1}{3}$ *ounce* $CaHPO_4$ *daily.*)

The *average weight* of these 8 animals at the commencement of the experiment was 437 lb. (highest 546, lowest 334), i.e. a few pounds lower than the average weight of the preceding groups.

The *rate of growth* is indicated by curve 6 in Figure 2. It will be seen that this curve keeps below curves 4 and 5. Attention may here be directed to Figure 5 in which curve 6 is compared with curve 3 ($\frac{1}{2}$ ounce bonemeal) and curve 2 (1 ounce bonemeal), respectively. It will be noticed that the weight curve of Group 6 is markedly better than that of Group 3, and is almost identical with that of Group 2. Curves 6 and 2 run parallel and very close together throughout the experiment; curve 6 starts slightly below curve 2 and ends up about the same distance below.

The average weight at the *conclusion of the experiment* of the 5 animals which had survived was 913 lb. (highest 978, lowest 820), an increase of 108.9 per cent over the initial weight.

The dose of phosphorus given to this group was insufficient to *prevent pica*. Pica curve 6 in Figure 2 shows that the percentage of bone eravers in this group remained high. During the latter period of the experiment the percentage remained above 70 per cent.

The inadequacy of the phosphorus ration was emphasized by the death of two of the 8 animals from lamsiekte. One died on the 6th December, 1928, and the other on the 31st August, 1929. A third animal died on the 15th October, 1929, just before the conclusion of the experiment, but in this case poisonous plants seem to have been the cause of death.

Groups 7 and 8.

(Controls, no phosphorus ration).

Group 7 consisted of 8 animals, as in the preceding groups. Their *average weight* at the beginning of the experiment was 439 lb. (highest 485, lowest 323), i.e. almost exactly the same as in the other groups.

Group 8 was added a few weeks later so as to have a larger number of controls, in case some should die of lamsiekte. The 6 animals in this group were slightly lighter than those in the other groups. Their *average weight* was 410 lb. (highest 448, lowest 373).

The *weight curves* of these two groups have been kept separate so as to facilitate an accurate comparison with the other groups (see curves 7 and 8 in Figure 6). Strictly speaking, only group 7 should be compared with the other groups, since the initial average weights in all these groups were practically identical, whereas that of group 8 was slightly lower; but it will be noticed that groups 7 and 8 behaved almost identically, their weight curves remaining parallel throughout the experiment. The lower initial weight of the animals in group 8 does not, therefore, seem to invalidate the comparison between all the controls and the phosphorus groups.

Figures 1 and 2 show clearly how much more rapid the growth of the animals in the phosphorus groups was than of those in the control groups. These latter remained far below the others and at the *conclusion of the experiment* the average weight of the 6 animals remaining in group 7 was 627 lb. (highest 700, lowest 470), an increase of 42.8 per cent. over the initial average weight; and the average weight of the 4 animals remaining in Group 8 was 583 lb (highest 610, lowest 546), an increase of 42.2 per cent. over the initial average weight.

As was to be expected, the *degree of pica* in the two control groups remained high throughout the experiment. In Group 7 the pica curve seldom came below 100 per cent., and in Group 8 seldom below 80 per cent.

In the course of the experiment 4 animals died of lamsiekte in these groups, 2 in Group 7, and 2 in Group 8. Deaths occurred in Group 7 on the 15th October, 1928 and 21st August, 1929, and in Group 8 on the 5th April, 1929 and the 9th April, 1929.

DISCUSSION OF RESULTS.

1. Influence of Phosphorus on Growth.

The most striking result in the foregoing experiments is again *the extraordinary stimulating influence of phosphorus on the growth of cattle* grazing on phosphorus deficient pasture. This influence is very clearly illustrated in Figure 1 which shows that the rate of growth is directly proportional to the amount of phosphorus given to each animal. Group 1 which received 3 ounces bonemeal daily increased in weight at a much more rapid rate than Group 2 which received 1 ounce; and Group 2 again increased much more rapidly than Group 3 which received only $\frac{1}{2}$ ounce. The percentage increase in weight of Group 1 at the conclusion of the experiment was 134 or about 24 per cent. more than that of Group 2 (110 per cent.), and this again was about 22 per cent. more than that of Group 3 (88 per cent.). This latter group, in its turn, showed an increase of more than double that of the controls which received no bonemeal (42 per cent.).

Naturally, there is a limit to this rate of growth; as soon as the daily ration of phosphorus reaches a certain *optimum*, no further increase in weight will be achieved by increasing the ration still further. Any phosphorus in the diet, above the optimum requirement of the animal, will be treated by the animal body as *excess* and will either be passed out unabsorbed or, if absorbed, will not be utilized in the demonstrable form. In other words, if an animal, or preferably, a group of animals should receive the optimum amount of phosphorus in their daily ration and another similar group double this amount, the weight curves of the two groups will probably be practically identical, although the latter group would pass out a much larger amount of phosphorus in their excretions.

This probably explains the similarity between the weight curves of Group 4 and Group 5 (see Figure 2). The former received 2 ounces calcium phosphate per head per day, whereas the latter received only $\frac{2}{3}$ ounce, i.e. one-third the amount; and yet their weight curves practically coincide. On the other hand, Group 6 which received $\frac{1}{3}$ ounce per day increased in weight at a much slower rate than Group 5 ($\frac{2}{3}$ ounce).

At the conclusion of the experiment Group 4 had increased about 127 per cent. in weight and Group 5 124 per cent. This small difference is probably of no significance; as a matter of fact, as was pointed out previously, Group 5 actually weighed more than Group 4 a month before the experiment terminated.

We may take it, therefore, that the $1\frac{1}{3}$ ounce calcium phosphate which the animals of Group 4 received in addition to the $\frac{2}{3}$ ounce which those in Group 5 received, had no appreciable effect and might be regarded as excess.

The ration which Group 5 received, namely $\frac{2}{3}$ ounce calcium phosphate daily, is probably very near the optimum requirements of cattle of this age. The optimum cannot be much lower because, when the ration was further reduced to $\frac{1}{3}$ ounce (Group 6), the rate of growth was very appreciably retarded (see Figure 2 and Table 1).

2. Influence of Phosphorus on Osteophagia.

The method of testing for the presence of Osteophagia or Pica in the past was not very accurate. An animal receiving a small ration of phosphorus might be just on the borderline of pica; at one test it might not take a bone and at the next test it might do so. In the one case it would be recorded as a non-craver and in the other as a craver. Expressed in figures, the difference would be as between 0 per cent. and 100 per cent.; whereas in reality the difference might not be more than 5 per cent. Tests are now being devised which will indicate the degree of osteophagia with much greater accuracy. But in the meantime we have to rely on the results obtained with the old tests.

The *degree of pica* in the various groups is indicated in Figures 1 to 6 in the form of "curves."

Figure 1 shows the influence of the various rations of bonemeal on pica. In Group 1 the pica was suppressed fairly successfully with 3 ounces of bonemeal, but in Group 2 the ration of 1 ounce of bonemeal seems to have had but little effect in preventing pica, and the ration given to Group 3 ($\frac{1}{2}$ ounce bonemeal) had almost no effect. The pica in this latter group was only slightly less than in the controls (Group 7).

In the calcium phosphate groups (Figure 2) the degree of pica was, on the whole, much lower than in the bonemeal groups. In Group 4 (2 ounces calcium phosphate) the pica was kept very low, somewhat lower than that in Group 1 (3 ounces bonemeal). Group 5 ($\frac{2}{3}$ ounce calcium phosphate) was again very similar to Group 4, whereas Group 6 ($\frac{1}{3}$ ounce calcium phosphate) showed a much higher degree of pica than either of these two groups.

For reasons explained above it is difficult to draw an accurate comparison between the different groups in regard to pica, but perhaps a fair indication may be obtained by comparing the total number of times the individual animals in each group picked up and chewed a bone in the tests. During the two years that the experiment lasted, pica tests were conducted on 53 occasions (at fortnightly intervals). If now every one of the 8 animals in a group had chewed a bone at every test, the total number of positive entries would have been 424. (The necessary correction would have to be made if an animal died in a group).

Calculating on this basis we find that:—

Group	1	showed	69	positive	tests	out	of	424	= 16.3 per cent.
,,	2	,,	203	,,	,,	,,	,,	424	= 47.9 per cent.
,,	3	,,	318	,,	,,	,,	,,	416	= 76.4 per cent.
,,	4	,,	50	,,	,,	,,	,,	424	= 11.8 per cent.
,,	5	,,	75	,,	,,	,,	,,	424	= 17.7 per cent.
,,	6	,,	207	,,	,,	,,	,,	397	= 52.1 per cent.
,,	7	,,	366	,,	,,	,,	,,	392	= 93.4 per cent.
,,	8	,,	224	,,	,,	,,	,,	282	= 79.4 per cent.

The influence of phosphorus on pica is clearly illustrated by these figures. In Groups 1, 2 and 3 the "percentage of pica" rises from about 16 to 48 to 76; and in Groups 4, 5 and 6 it rises from about 12 to 18 to 52. The percentage figure for the two groups of controls is 87.5.

3. Comparison between Bonemeal Groups and Calcium Phosphate Groups.

When the experiment was planned the idea was that Group 1 would be compared with Group 4, Group 2 would be compared with Group 5, and Group 3 would be compared with Group 6.

If we compare the groups in this way, the calcium phosphate groups show up to much greater advantage than the bonemeal groups.

To facilitate comparison the outstanding results of the experiment have been summarized in the following table:—

Group No.	Phosphorus Ration (Daily).	Initial Average Weight. (19/10/27).	Final Average Weight. (2/11/29).	Increase in Weight.	Percentage Increase.	Pica Percentage. *	Total Cost of Phosphorus Ration per Animal. †
		lb.	lb.	lb.	%	%	s. d.
1	3 oz. bonemeal....	441	1,032	591	134	16·3	9 7
2	1 oz. bonemeal....	439	923	484	110·3	47·9	3 2½
3	½ oz. bonemeal....	439	827	338	88·4	76·4	1 7
4	2 oz. $CaHPO_4$....	439	1,000	561	127·8	11·8	9 11½
5	2/3 oz. $CaHPO_4$...	440	986	546	124·1	17·7	3 4
6	1/3 oz. $CaHPO_4$...	437	913	476	108·9	52·1	1 8
7	—	439	627	188	42·8	93·4	—
8	—	410	583	173	42·2	79·4	—

Comparing first of all Group 3 (½ ounce bonemeal) with Group 6 (⅓ ounce calcium phosphate), we see that the latter is superior in every way to the former (see Figure 5). The increase in weight is much greater and the degree of pica much lower. In other words ⅓ ounce calcium phosphate is much more effective than ½ ounce bonemeal.

As a matter of fact, group 6 compares very favourably with Group 2 (1 ounce bonemeal). (See Figure 5.) The increase in weight is practically identical and the degree of pica in the two groups is almost the same. We may conclude that ⅓ ounce calcium phosphate has practically the same value, both as a growth stimulant and as a remover of pica, as 1 ounce bonemeal. It would seem that calcium phosphate is about three times as effective as bonemeal.

Comparing now Group 2 (1 oz. bonemeal) with Group 5 (⅔ ounce calcium phosphate) we find the same difference in favour of the calcium phosphate group as we saw in the comparison of Groups 3 and 6. Group 5 increased in weight much more rapidly and shows far less pica than group 2 (see Figure 4.)

The comparison between groups 1 (3 ounces bonemeal) and 4 (2 ounces $CaHPO_4$) is rendered difficult by the fact that in both cases the animals probably received an *excess* of phosphorus. Growth

* Calculated in the manner described on page 275.

† Reckoning bonemeal at £8 per ton, and calcium phosphate at £12. 10s. per ton, and calculating to the nearest halfpenny.

in Group 1 was slightly more rapid than in Group 4; on the other hand the degree of pica was less in the latter group than in the former. (See Figure 3.)

It has been pointed out above that Groups 4 and 5 (2 ounces and $\frac{2}{3}$ ounce calcium phosphate respectively) behaved very similarly. Increase in weight in the two groups was practically the same and pica was only very slightly worse in Group 5.

It is of interest, therefore, to compare Group 5 with Group 1. The rate of increase in weight was slightly better in Group 1, but from the point of view of pica prevention these two groups were almost identical.

We see, therefore, that a ration of $\frac{2}{3}$ ounce calcium phosphate had almost the same effect as 3 ounces bonemeal ($4\frac{1}{2}$ times its weight). Of course, it must be remembered that 3 ounces of bonemeal (as pointed out before) probably represent an "excessive" dose. It is quite possible that a somewhat smaller dose (say 2 or $2\frac{1}{2}$ ounces) would have given almost the same results as 3 ounces. However, we know from previous experiments that the optimal dose of bonemeal for the class of animal used in this test would not have been lower than 2 ounces. If now we assume that 2 ounces of bonemeal would have been almost as effective as 3 ounces and would have compared favourably with $\frac{2}{3}$ ounce calcium phosphate, we again arrive at the conclusion that calcium phosphate is apparently three times as effective as bonemeal.

It is not easy to explain this superiority of calcium phosphate. It is true that this compound contains about 42 per cent. P_2O_5, whereas bonemeal usually has about 22 per cent., but this difference does not fully explain the very much superior results obtained with equivalent amounts of calcium phosphate. It would seem that the finer physical state of the calcium phosphate powder facilitates its absorption by the animal and that, therefore, its phosphorus is more readily available than that contained in bonemeal.

Furthermore, these two products are chemically different, of course; practically all the phosphorus in calcium phosphate is soluble in 2 per cent. citric acid, whereas only about 50 per cent. of that in the bonemeal used is soluble. This again would suggest greater availability of the phosphorus in calcium phosphate than that in the bonemeal.

4. The Cost of Phosphorus Feeding.

The question was put at the beginning of this paper "*What is the effective dose of calcium phosphate and how does the price of this dose compare with the price of the effective dose of bonemeal?*"

In the experiments described above it was shown that $\frac{1}{3}$ ounce calcium phosphate was quite ineffective to produce maximal growth or prevent pica, whereas $\frac{2}{3}$ ounce seemed to satisfy the animal's requirement of phosphorus.

Similarly, 1 ounce of bonemeal was quite insufficient, whereas 3 ounces produced excellent results.

For the removal of pica $\frac{2}{3}$ ounce of calcium phosphate was as effective as 3 ounces of bonemeal, and for stimulating growth it was only slightly less effective. It would be of interest therefore to compare the cost of these two quantities.

The bonemeal used in the experiment was bought at about £8 per ton, and the calcium phosphate was supplied at £12. 10s. per ton. Three tons of the former products would therefore cost £24 and two-thirds of a ton of calcium phosphate £8. 3s. 4d. Hence the cost of the former quantity is very nearly *three times* that of the latter. The relative costs of 3 ounces of the former and two-thirds of an ounce of the latter would, of course, be in the same proportion. Even assuming that 2 ounces of bonemeal would have been as effective as 3 ounces, the cost of the former dose would have been about twice that of $\frac{2}{3}$ ounce of calcium phosphate.

The above comparison would naturally only remain valid so long as the prices of bonemeal and calcium phosphate remained in the same proportion as that quoted above. The price of bonemeal has been fairly constant for years, but the price of calcium phosphate is uncertain. The article supplied to us was imported and was manufactured from bones; attempts are now being made by local firms to manufacture it in South Africa from rock phosphates. If these attempts should prove to be successful the price of calcium phosphate may be brought still lower.

Another advantage of calcium phosphate in comparison with bonemeal, is the smaller quantity needed for the same number of animals and the consequent saving in the cost of transport—a very important item for farmers in the remoter parts of South Africa.

And a further advantage is the ease of administering the smaller dose to the animals.

For interest, the cost of the total amount of the supplementary ration fed to each animal during the two years that the experiment lasted, has been calculated and entered in the last column of the table on page 276. Attention may be directed to the fact that the additional 358 pounds which the animals in Group 5 added to their weight in comparison with the controls, were obtained at a cost of 3s. 4d. or about one-ninth of a penny per lb.!

The nature of the supplementary phosphorus ration to be given in the future to cattle in South Africa, will be determined by economic factors. Price and efficacy will decide whether the ration will be in the form of a calcium phosphate or some other phosphorus compound. Experiments are now proceeding with various compounds to ascertain whether optimum results cannot be obtained with a product which will be cheaper still than calcium phosphate.

SUMMARY AND CONCLUSIONS.

(1) An experiment is described in which dicalcium phosphate ($CaHPO_4$) and bonemeal were given, in varying amounts, to cattle grazing on phosphorus deficient veld, in order to test the relative value of these two compounds as a phosphorus supplement. Sixty-two cattle were used in the experiment which continued over two years.

2. The cattle were divided into seven uniform groups of 8 animals each; the seventh (control) group being subsequently increased by the addition of 6 more animals.

The seven groups received the following supplementary rations daily (except Sundays): (1) 3 ounces bonemeal, (2) 1 ounce bonemeal, (3) $\frac{1}{2}$ ounce bonemeal, (4) 2 ounces calcium phosphate, (5) $\frac{2}{3}$ ounce calcium phosphate, (6) $\frac{1}{3}$ ounce calcium phosphate, (7) and (8) no phosphorus ration (controls).

3. At the conclusion of the experiment the value of the supplementary ration was judged by—

(*a*) its effect on the *growth* (*weight*) of the cattle; and

(*b*) its effect on the *pica* of the cattle.

Furthermore, the relative *cost* of the rations was examined. In the table on page 278 the outstanding results have been summarized.

4. It was found that 3 ounces of bonemeal produced a very marked increase in weight (134 per cent.), 1 ounce much less (110 per cent.), and $\frac{1}{2}$ ounce much less still (88 per cent.). In the calcium phosphate groups 2 ounces produces a very marked increase in weight (128 per cent.), $\frac{2}{3}$ ounce almost the same (124 per cent.), and $\frac{1}{3}$ ounce much less still (109 per cent.). The weight of the control animals increased by only 42 per cent.

5. For the *prevention of pica* 2 ounces of calcium phosphate proved to be most effective, although only slightly more so than 3 ounces bonemeal or $\frac{2}{3}$ ounce calcium phosphate which had about the same effect. One ounce bonemeal and $\frac{1}{3}$ ounce calcium phosphate were both much less effective than the larger doses. Half an ounce of bonemeal had almost no effect on the craving for bones.

6. In analysing the results the conclusion was reached that two-thirds of an ounce of calcium phosphate practically satisfied the phosphorus requirement of the animals, and probably represented the optimum dose. The optimum dose of bonemeal could not be determined with accuracy, since 3 ounces were probably in excess of the optimum and 1 ounce definitely below it. It was concluded that the optimum dose for the class of animal used was certainly not below 2 ounces. This would seem to show that, weight for weight, *calcium phosphate is three times as effective as bonemeal.* The same conclusion is reached when Group 6 ($\frac{1}{3}$ ounce calcium phosphate) is compared with Group 2 (1 ounce bonemeal.)

7. It is difficult to draw definite conclusions in regard to the *relative cost* of the two compounds, since the ultimate cost will depend on the demand for the product, the source, the process of manufacture, etc. But if, meanwhile, the prices paid for the products used in the above experiment, are taken, it is found that *the effective dose of bonemeal is at least twice as expensive as the effective dose of calcium phosphate.*

8. Our final conclusion is that, as a supplementary ration for cattle grazing on phosphorus deficient veld, precipitated dicalcium phosphate is a more effective and relatively much cheaper source of phosphorus than bonemeal.

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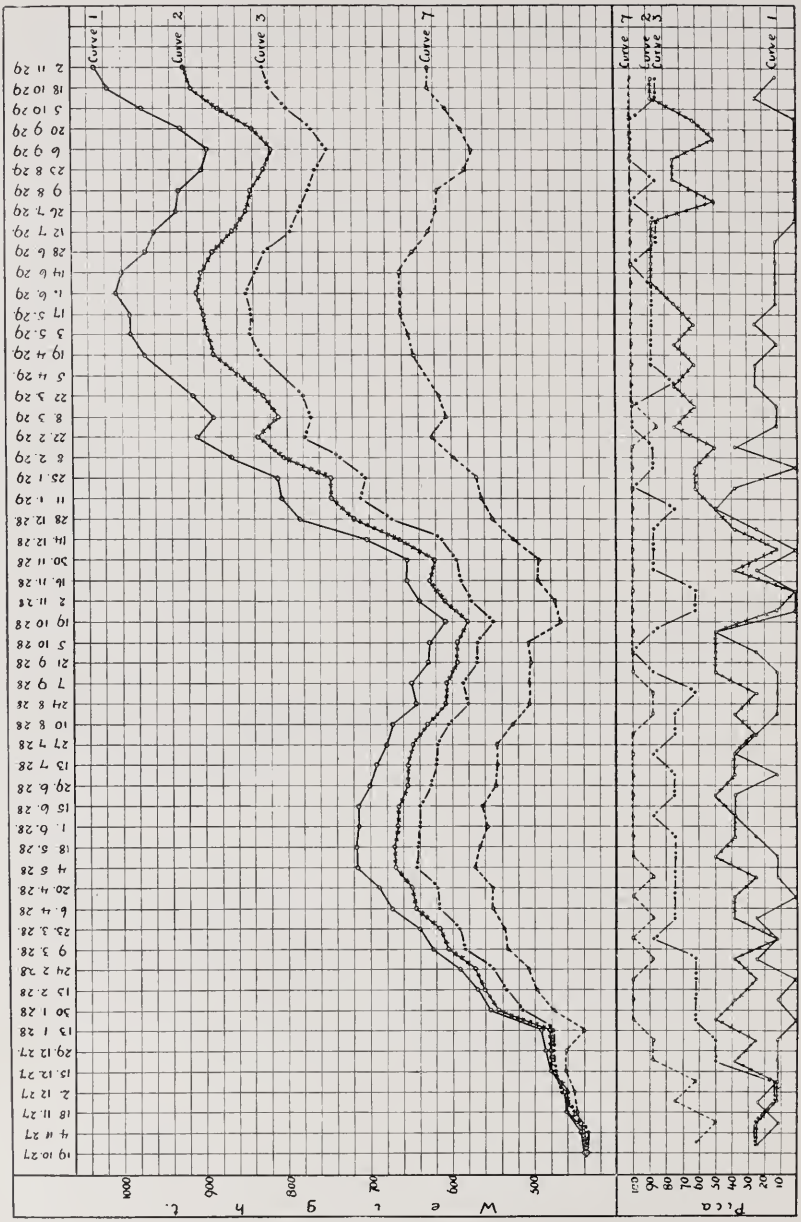
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Curve 3
Curve 7
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22 3 29
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17 5 29
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FIG. 1.

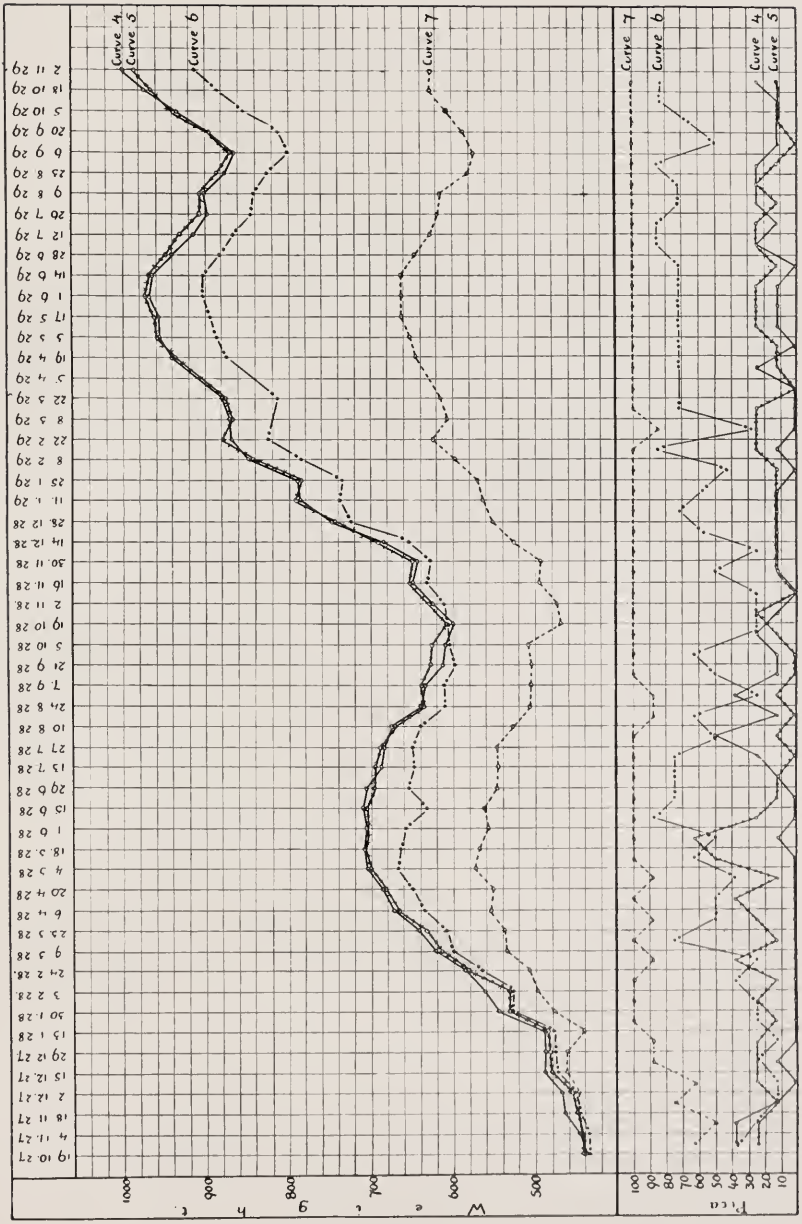
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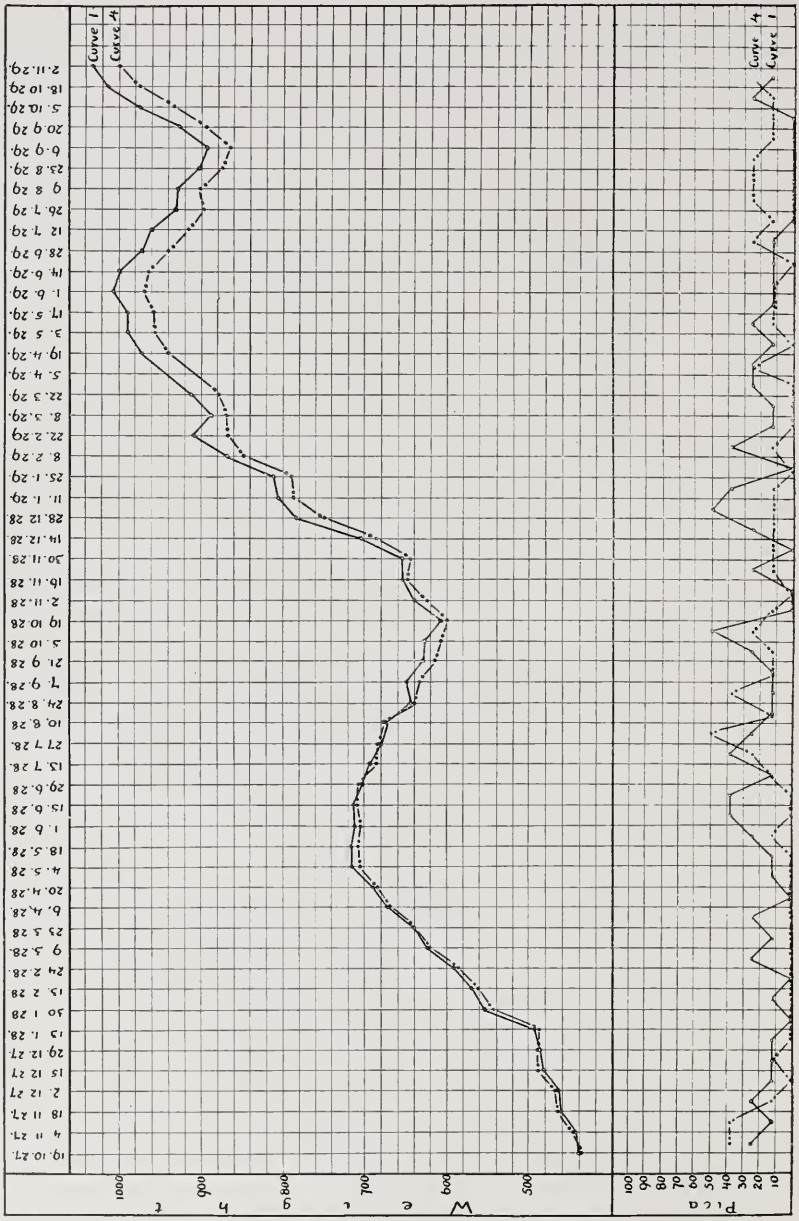
FIG. 2.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Curve 4
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FIG. 3.

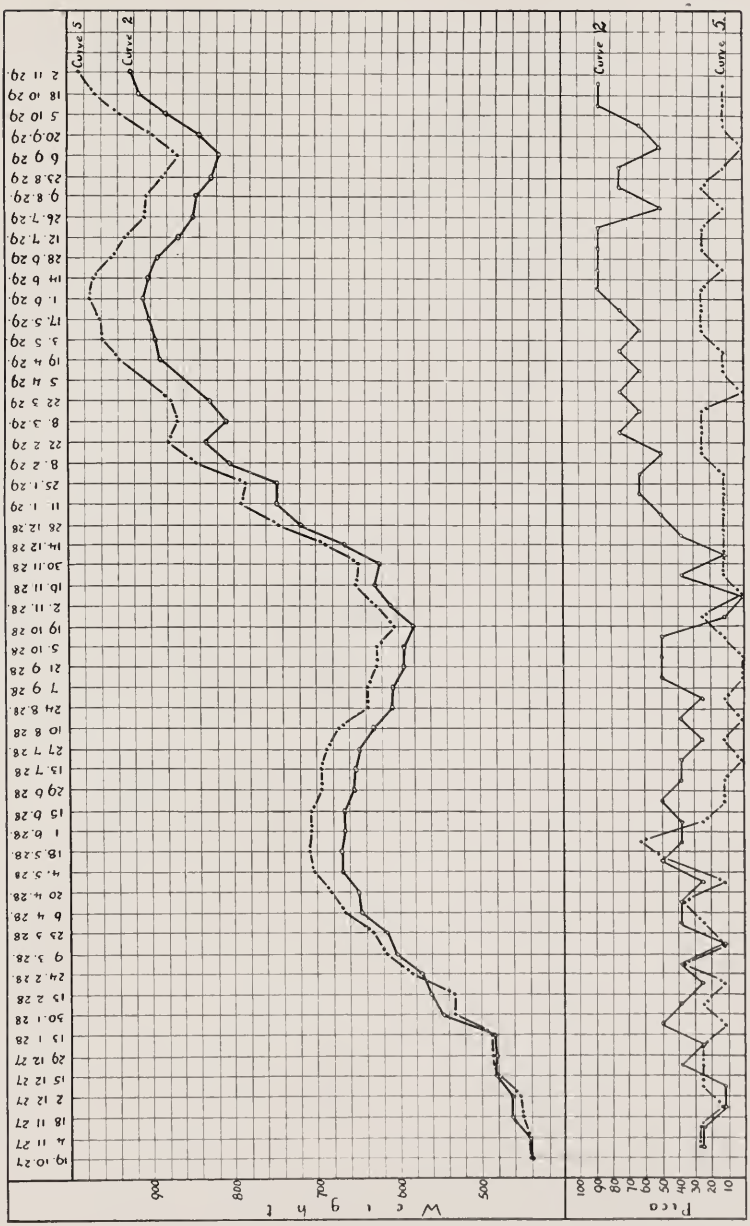
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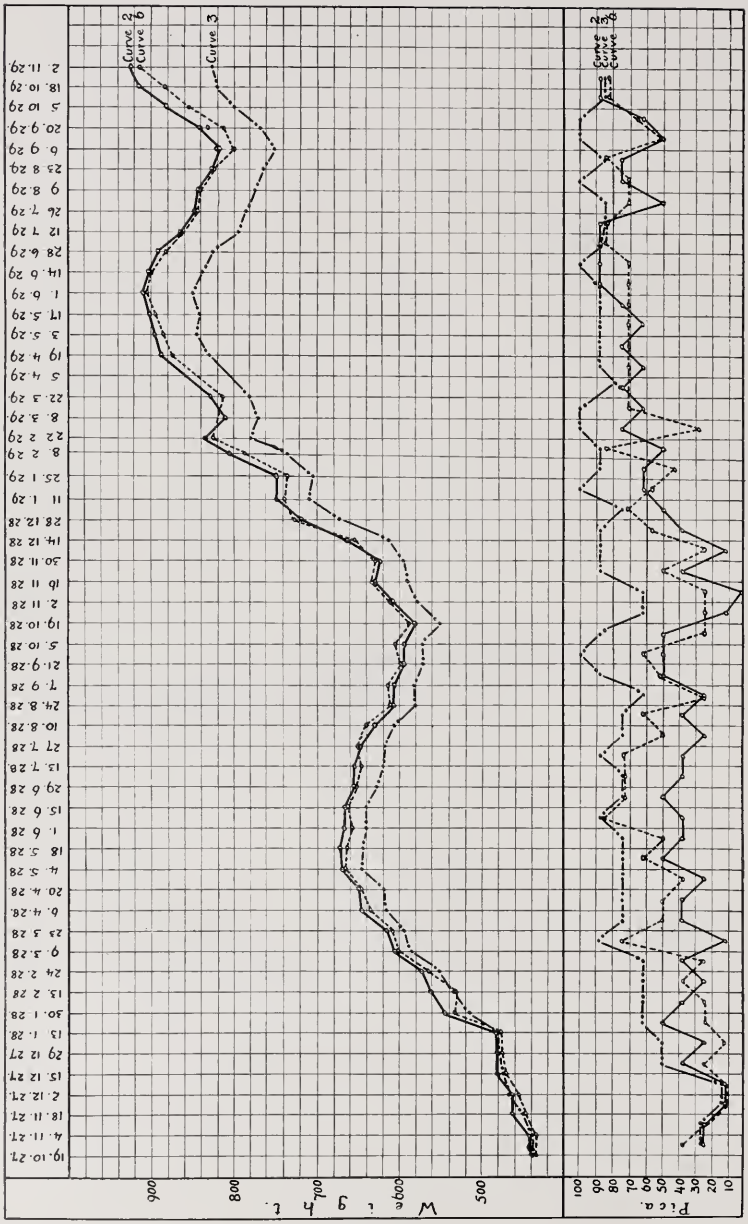
Fig. 4.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Curve 2
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FIG. 5.

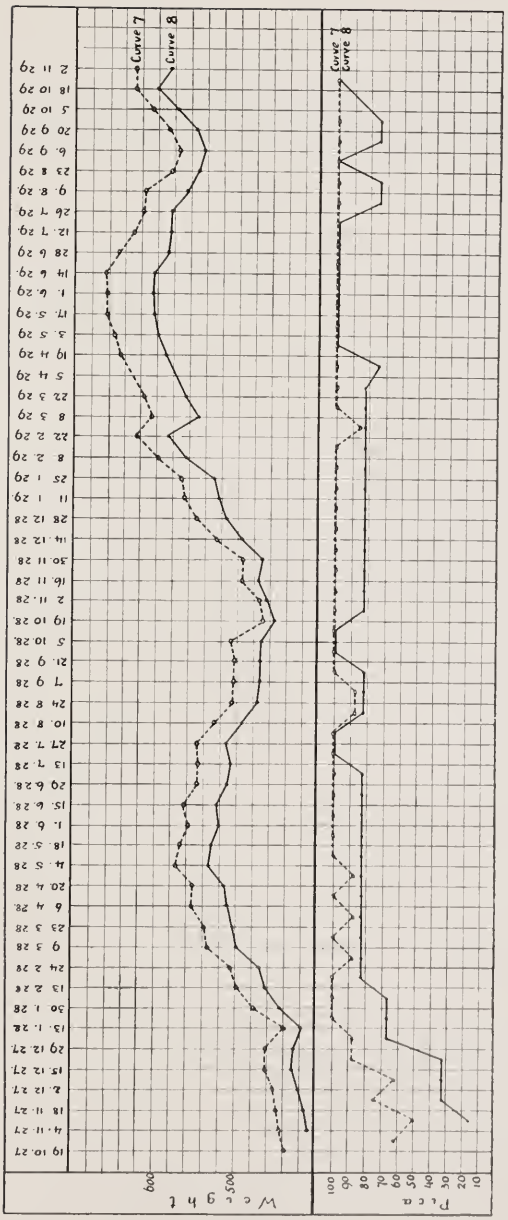
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Fig. 6.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

The Increase of Inorganic Phosphorus Due to Hydrolysis in Solutions of Blood.

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If Blood be allowed to stand the inorganic phosphorus gradually increases due to hydrolysis of the organic acid soluble phosphorus fraction. The question has been studied by many workers and no attempt will be made to go into the literature as the results of the findings are more or less common knowledge and have brought about modifications in the customary methods of estimating inorganic blood phosphorus.

The present study arose out of the need to send blood samples from various parts of the Union of South Africa to Onderstepoort, a journey, with any delay, that might take a week. These samples were for the purpose of analyses for inorganic phosphorus. Then too there has been the desire to determine at Onderstepoort the inorganic phosphorus in the blood of cattle suffering from " mudu " in Nigeria. There is a strong suspicion from the analysis of grasses and soil that have been sent here for analysis that " mudu " might be aphosphorosis. It became necessary, therefore, to work out a system of keeping blood solutions for periods up to approximately three weeks so that the inorganic phosphorus fraction on analysis would still indicate whether the animal from which the blood was drawn suffered from phosphorus deficiency or not, as low inorganic blood phosphorus is indicative of aphosphorosis as suggested by Green (1927) and definitely shown to be the case by Malan (1930).

The method of treating the blood without disturbing its inorganic phosphorus appreciably was determined by what could be done under field conditions, as obviously men in the field would draw the blood and despatch it, after treatment, to the central station. Adding a number of preservatives such as toluol, chloroform and phenol, was tried with unsatisfactory results, but this method is far from being exhausted. For the precipitation of the blood proteins an extremely simple field outfit would be necessary, for it only means drawing the blood, transferring a definite quantity with a pipette to a bottle containing the necessary amounts of precipitant and forwarding the resulting mass to Onderstepoort.

As a result of these considerations the hydrolysis factor was determined by filtering off the precipitated blood proteins after allowing them to stand for different periods with precipitants and all and analysing the filtrates for inorganic phosphorus. " Precipitated mass," mentioned in the tables was treated in this manner. Further ,a field outfit comprising the necessary apparatus for the precipitation and filteration of the blood proteins is not very cumbersome and, therefore, the hydrolysis in filtrates was considered as well. Sheep, bovine and horse blood was used.

The usual procedure for the determination of phosphorus as described by Green (1928) was followed and the blood solutions kept for the periods indicated in the tables below:

TABLE I.

Inorganic Phosphorus in Mgm. per 100 *c.c. blood.*

Animal.	Source.	0 hrs.	24 hrs.	72 hrs.	6 days.	21 days.	Percentage Increase.
Horse.....	Blood filtrate.....	2·9	3·2	3·2	3·4	4·0	38·0
„	„ „	4·5	4·6	4·6	4·9	5·3	17·7
Bovine.....	„ „	7·1	7·3	7·4	7·8	8·2	15·5
Sheep.....	„ „	5·8	6·4	6·6	7·1	7·5	29·3
Bovine No. 2673.	Blood filtrate....	4·0	4·0	4·6	4·8	4·9	22·5
	Precipitated mass	4·0	4·0	4·6	—	4·9	22·5
Bovine No. 2674.	Blood filtrate....	7·6	7·6	8·6	8·7	8·9	17·1
	Precipitated mass	7·6	7·6	8·4	8·5	8·7	14·5
Bovine No. 2681.	Blood filtrate....	7·0	7·0	7·7	—	8·2	17·2
	Precipitated mass	7·0	7·0	7·7	7·7	7·9	12·8

The final concentration of the precipitant Trichloracetic acid in the determination given above was 5 per cent. The hydrolysis is quite appreciable. In the next table 2.5 per cent. trichloracetic acid solution was used.

TABLE II.

Inorganic Phosphorus in Mgm. 100 *c.c. blood.*

Animal.	Source.	0 hrs.	24 hrs.	72 hrs.	8 days.	21 days.	Percentage Increase.
Bovine No. 2673.	Filtrate.....	4·0	4·0	4·2	4·2	4·2	5·0
	Precipitated mass	4·0	4·0	4·0	4·0	4·0	0
Bovine No. 2674.	Filtrate.....	7·6	7·6	7·9	7·9	8·1	6·5
	Precipitated mass	7·6	7·6	7·9	7·9	7·9	3·9
Bovine No. 2681.	Filtrate.....	7·0	7·0	7·2	7·5	7·5	7·2
	Precipitated mass	7·0	7·0	7·2	7·2	7·2	2·9
Bovine No. 3526.	Precipitated mass	5·9	—	—	5·9	—	0
„ „ 3502.	„ „	7·6	—	—	7·6	—	0
„ „ 3524.	„ „	6·9	—	—	6·9	—	0
„ „ 3522.	„ „	8·2	—	—	8·2	—	0
„ „ 3544.	„ „	7·6	—	—	7·6	—	0
Sheep No. 1.....	Precipitated mass	6·1	—	—	6·3	—	3·3
„ „ 2.....	„ „	6·5	—	—	6·8	—	4·6
„ „ 3.....	„ „	4·4	—	—	—	—	—
„ „ 4.....	„ „	3·2	—	—	3·2	—	—

Table III.

Inorganic Phosphorus in Blood of Sheep.

No.	True Value.	Filtrates kept for 8 Days.	Percentage Increase.	Precipitated mass kept for 8 Days.	Percentage Increase.
23964	3·9	4·6	17·9	3·9	0
23965	3·5	4·2	20·0	3·8	8·6
24006	2·3	3·0	30·0	2·6	13·0
23968	3·2	3·9	21·9	4·0	25·0
23969	3·3	3·7	12·1	3·3	0
23970	3·2	3·5	9·4	3·7	15·6
23971	3·6	4·0	10·4	4·0	10·4
23972	3·4	3·9	14·7	3·7	8·8
23973	3·9	5·0	28·2	—	—
Average	3·4	3·9	14·7	3·6	5·9
23974	3·9	5·1	30·8	4·8	23·0
23975	4·2	5·0	19·0	4·7	11·9
23976	3·7	4·1	10·8	3·7	0
23977	4·6	5·3	15·2	4·9	6·5
23978	5·2	—	—	5·3	1·9
23979	3·7	4·0	8·1	—	—
23980	3·4	4·2	22·8	4·2	22·4
23981	4·5	—	—	5·2	15·5
23982	5·1	5·8	13·7	—	—
23983	5·5	6·2	12·7	6·2	12·7
Average	4·4	5·0	13·6	4·9	11·3
23984	5·8	·66	13·8	—	—
23985	9·0	9·2	22·2	—	—
23986	8·3	9·4	13·2	9·5	14·6
23987	8·5	9·3	9·4	9·0	5·9
23988	6·9	7·5	8·7	7·7	11·6
23989	7·4	8·9	17·6	8·3	12·2
23990	9·1	9·7	6·6	9·5	4·4
23991	7·1	7·5	5·7	7·5	5·7
23992	6·8	7·1	4·4	7·4	8·8
23995	7·9	8·7	10·1	8·9	12·7
23996	7·9	8·0	1·3	8·1	2·6
Average	7·7	8·4	9·1	8·4	9·1
23964	2·4	4·6	91·8	4·7	96·0
23965	2·9	4·4	51·9	—	—
24006	2·0	3·4	70·0	4·0	100·0
23968	2·6	3·8	46·2	3·9	50·0
23969	3·0	4·4	46·6	4·8	60·0
23970	2·7	3·8	40·9	4·2	55·6
23971	3·1	4·6	48·5	4·7	51·6
23972	2·6	3·6	38·5	4·3	65·4
23973	2·9	—	—	4·2	44·9
Average	2·7	4·1	51·9	4·3	59·2

Inorganic Phosphorus in Blood of Sheep. Table III (cont.).

No.	True Value.	Filtrates kept for 28 Days.	Percentage Increase.	Precipitated Mass kept for 28 Days.	Percentage Increase.
23974.....	4·9	6·0	22·5	5·5	12·2
23975.....	5·5	6·5	18·2	6·2	12·7
23976.....	5·0	6·3	26·0	6·0	20·0
23977.....	3·1	4·3	38·7	3·8	22·5
23978.....	4·8	5·8	20·0	6·1	27·1
23979.....	5·6	6·5	16·1	6·6	17·9
23980.....	3·2	4·4	37·5	4·9	53·0
23981.....	3·8	5·2	36·8	5·4	42·1
23982.....	3·3	4·8	45·5	5·0	51·6
23983.....	4·8	5·6	16·7	5·0	4·2
Average.....	4·4	5·5	25·0	5·4	22·8
23984.....	4·8	6·0	25·0	5·9	22·9
23985.....	8·1	9·8	21·0	9·7	19·8
23986.....	5·2	6·6	26·9	7·5	44·2
23987.....	8·8	9·4	68·1	10·2	15·9
23988.....	8·1	11·7	44·5	9·7	19·7
23989.....	7·2	8·5	18·1	7·5	4·2
23990.....	5·7	7·2	21·1	7·4	29·8
23991.....	10·7	21·1	13·1	12·2	14·0
23992.....	9·8	—	—	—	—
23995.....	6·5	8·7	33·9	8·2	26·2
23996.....	7·5	9·6	28·0	9·7	29·3
Average.....	7·5	9·0	20·0	8·8	17·4

DISCUSSION.

The outstanding feature of the results is a confirmation of what one might suspect, viz., that hydrolysis proceeds more slowly in a 2.5 per cent. trichloracetic acid solution than in a 5 per cent. solution. What is, however, of interest and of practical value in cases where blood takes time to reach the central station is the fact that precipitation of the blood proteins without filtration decreases the rate of hydrolysis of the organic acid soluable phosphorus into inorganic phosphorus very considerably. This is shown clearly in Tables I and II. In the latter case 5, 6.5 and 7.2 are the percentage increases in inorganic phosphorus of three blood filtrates after 3 weeks' standing while the corresponding figures for the precipitated masses for the same period are 0, 3.9 and 2.9 respectively. Table III illustrates the same point for a fairly large number of sheep. In one case, however, the precipitated mass showed a slightly larger increase than when the filtrates of the same blood was kept. This happens to be after hydrolysis continued for 28 days, a period too long in any case for this work if the blood samples from Nigeria be not considered at present. Eight days and maybe fourteen days show little increase in inorganic phosphorus of the precipitated mass; the increase is less for the precipitated mass than for the filtrates in all cases but two, where the same values were obtained. Considering the ease with which precipitated blood solutions can be obtained and forwarded

compared with completing the process of filtering in the field it is rather fortunate that hydrolysis proceeds more slowly in the precipitated mass.

The irregularity of the increase of inorganic phosphorus for different animals is striking, but as this does not affect the problem under consideration, further work on that particular aspect must be left until later. The quantity of the organic acid soluble phosphorus present is most probably a contributing factor.

From the point of view of field work, it is obviously the better method to use 2.5 per cent. trichloracetic acid solution for the precipitation of the proteins and to forward the precipitated mass to the central station, the filtration being left until immediately before the determination of inorganic phosphorus. It seems that horse, bovine and sheep blood can be treated successfully in this way and the precipitated mass delayed on a journey for approximately eight days without making the interpretation of the results impossible. From the large number of samples analysed the highest percentage increase of inorganic phosphorus during 8 days was 25.0 although it must be pointed out that the average increase is only about eight per cent. which does not affect the original figure appreciably. This becomes clearer by referring to Table III which gives the inorganic phosphorus content of the blood of 31 sheep that provided excellent material for this work as they were being kept on known phosphorus rations in connection with another problem. The first ten sheep are on a diet as deficient in phosphorus as a basal ration, poor in minerals, could produce. The average inorganic phosphorus of the blood of these sheep is 3.4 while the precipitated mass showed a figure of 3.6 after 8 days. The latter figure would enable one to diagnose phosphorus deficiency without the slightest hesitation. The next batch of 10 sheep shows 4.4 and 4.9 mgm. for inorganic blood phosphorus representing respectively the true value and that obtained after keeping the precipitated mass for 8 days. Both are average figures. Those for the third group are definitely high and the animals in that group are on a ration containing excess phosphorus.

It must be emphasized that the author does not wish to infer that hydrolysis, after the blood solutions have been kept for 8 days, is negligible but that the figure, although proportionately higher, still enables one to gauge the degree of aphosphorosis of the donors fairly accurately. Further, if blood solutions of at least ten animals—bovines by preference as the hydrolysis is apparently least in that case—be sent in from the field and the journey does not last longer than about eight days, the average figure for inorganic phosphorus is as accurate a reflection of the phosphorus intake of the animals concerned as one could wish for under field conditions. In any case such figures coupled with the phosphorus content of the grass and soil from the same area will yield valuable data on the problem of phosphorus deficiency in the Union of South Africa and perchance be of assistance in rectifying this deficiency.

SUMMARY.

1. The increase in inorganic phosphorus in blood when kept after precipitation of the proteins without filteration for different periods up to 28 days has been studied.

2. Hydrolysis is less in a 2.5 per cent. trichloracetic acid solution than in a 5 per cent. solution for the same period.

3. If the blood solutions are kept without filtering hydrolysis proceeds more slowly than when the filtrates are kept for the same time.

4. The suggestion is made that the hydrolysis which takes place in the precipitated mass even 8 days after precipitation of the blood proteins does not prevent the accurate estimation of the phosphorus deficiency or sufficiency of animals. Further, this enables one to carry out a phosphorus survey of large areas, especially if grass and soil be done at the same time.

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A Note on some Conditions in Sheep in the Strandveld of the Bredasdorp District.

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It will be of interest to record the occurrence of some peculiar conditions of sheep grazed on the Strandveld of the Bredasdorp District.

Sheep farming is the most important activity of farmers of this area. The veld has poor grazing value. The vegetation consists mainly of shrubs and bushes (South-western flora), with little grass. The carrying capacity for sheep is low, about 2 to 3 morgen are required for one animal. The soil is sandy in texture and very unfertile. Crops are raised with great difficulty unless fertilizers have been applied liberally.

How Sheep Farming is carried on in the Strandveld.

Most of the Strandveld farms are owned by farmers who also have farms in the more inland parts (called the Ruggens or Rûensveld). The Rûensveld is much more fertile and wheat is grown extensively. In general, sheep thrive on the Rûensveld. It is customary for these farmers possessing Rûensveld and Strandveld farms to interchange their sheep from the farms in the Strandveld to the Rûensveld and vice versa. Some time after the sheep have been on the Strandveld farms they become unthrifty and start dying from a disease called "Lamsiekte." A change becomes necessary and the sheep are moved back to the Rûensveld. This interchanging constantly goes on.

Sheep seem to stop growing as soon as they come on to the Strandveld and gradually lose condition. Ewes never seem to come into oestrum, and very few, if any, lambs are dropped on this veld. In cases where farmers own only Strandveld farms they buy sheep, usually year old lambs, from farmers on the Rûensveld. Their losses are constantly made good in this manner.

The cessation of further development and growth of sheep on these farms is forcibly brought out by the following weights of some sheep from this area.

Group I: Average live weight of 39 two-year old ewes—39.8 lb.

Group II: Average live weight of 39 one-year old lamb ewes—46.1 lb.

These two groups were bred from the same flock of ewes. Group I had been on the Strandveld for 14 months, whereas Group II had been on this veld for three months. It will be noticed that the ewes

fully a year younger are nearly 16 per cent. heavier than their older sisters. In other words, the sheep which were grazed on the Strandveld showed no further body development, but on the other hand, lost condition.

It is practically impossible to distinguish mature animals from the younger ones in a flock by their size and development. Sheep are kept on the Strandveld entirely for wool production; wool from this area is of a fine quality.

The undeveloped condition of the Strandveld sheep is very well illustrated on plate No. 1. The large wether is a full-mouthed sheep bred and kept on the Rûensveld while the smaller ones are wethers and ewes of the same age but kept on the Strandveld. These animals are all bred by the same owner and are of the same type of merinos.

PLATE No. 1.

THE SKELETAL SYSTEMS OF SHEEP GRAZED ON THE STRANDVELD.

As has been said, sheep of the Strandveld are markedly underdeveloped. The skeletal systems show well-defined abnormalities. The bones from such sheep are very light and break easily. This is realized by the farmers as they know from experience that these animals must be handled gently otherwise fractures of bones of the legs or ribs easily occur. A post-mortem examination of several of these animals was made, the softness of the bones encountered in such a dissection was quite remarkable. It was very easy to cut through these bones with an ordinary penknife, even the cranial cavity could be opened very easily. In many cases the incisor teeth were very long and lose.

Some bones from two animals which were grazed on this veld were collected. These bones were weighed, analysed and the average results compared with the same bones of two sheep of the same age and apparently normal skeletal development. The results of this examination are given in Table I.

TABLE I.

Analysis of Bones.

	Sheep from Bredasdorp.		Controls.	
	Femur.	Ribs.	Femur.	Ribs.
Specific gravity	·87	—	1·19	—
Total weight	24·1	9·3	80·7	37·1
Weight of ash	10·7	4·9	38·5	19·42
Percentage ash	46·6	52·5	47·7	53·0
Percentage P in bone	8·5	9·5	9·15	10·2
P_2O_5 in bone	19·5	21·6	21·0	23·4
Ca in bone	17·4	19·6	18·8	22·3
CaO in bone	24·4	27·5	26·8	31·6
Ca : P ratio	2·05	2·09	2·03	2·08

Except for the specific gravity and the total weight of the two sets of bones the results given in Table I do not show any appreciable difference between the bones of normal sheep and of sheep from the Strandveld area. This is in agreement with the results obtained by Evans (1930) for the bones of normal and rachitic swine. Although the bones are obviously different in physical properties, for instance those from the Strandveld are so brittle that they may be snapped very easily between the fingers, the Ca and P contents and ratios are apparently practically unaltered. It may yield interesting results to examine some bones histologically and mechanically. This the authors hope to include with X-ray examination in future work on the bones of animals on normal and on phosphorus deficient diets.

Fractures of long bones and ribs occur frequently. Plate No. 2 is a photograph of two ribs from an animal from this veld. Note the numerous calluses as the result of previous fractures.

A condition known as *Lamkruis* (paralysis of the lumbar region) is said to occur frequently amongst sheep of that area. Small game (duiker and steenbuck), which abound there, are said to suffer from the same condition. Unfortunately, the authors did not see a case, but from the description given by farmers this condition seems to result from a fracture of bones of the pelvis. The animals, as the name " lamkruis " indicates, are unable to raise their hindquarters and these are dragged when the animal is forced to move. Needless to say these sufferers are mercifully destroyed when found.

" LAMSIEKTE " OF SHEEP.

" Lamsiekte " is the most important disease of sheep of the Strandveld. It has been known for generations. Farmers are uncertain of the nature and the cause of this disease. Some maintain that it is caused by the animals drinking foul and stagnant water.

Several animals suffering from this condition were examined. They had been ill for different periods, varying from 3 to 6 days. The

PLATE No. 2.

most pronounced symptom was great weakness. In some cases the sheep were quite unable to stand and when picked up immediately fell down again. In other cases they were still able to walk a few paces, but the gait was uncertain and they soon collapsed. The animals which were very weak remained in the position in which they fell and were unable to assume the normal position of lying down. No other distinct clinical symptoms could be determined.

A careful post-mortem examination of two sick animals was made. No definite pathological changes were found in the various

systems. The carcasses in both cases were emaciated, but all the viscera seemed normal. Macroscopically the nervous system also appeared normal.

The general appearance of these sick sheep closely resembles cases of *Lamsiekte* (parabotulism) of cattle. The negative post-mortem findings is also on a par with Lamsiekte of cattle. Lamsiekte of cattle is very prevalent in the Bredasdorp district. In many instances the farmers of those parts regard it as a mysterious disease and are entirely ignorant of its cause, in spite of the fact that its etiology was clearly established some years ago. Their ideas of the nature and cause of this Lamsiekte in sheep must, therefore, be considered critically.

Osteophagia and Allotriophagia in Sheep.

It is stated by Theiler that osteophagia in sheep has not been observed in South Africa. On the Strandveld, however, sheep definitely show osteophagia and even allotriophagia. Carcass material is readily consumed by sheep on that veld.

Some bones were collected and strewn about. The sheep were then driven to this place; most of the animals deliberately started chewing these bones as shown on plate No. 3.

Plate No. 3.

If a dead sheep is left on the veld in that part the carcass is soon eaten by other sheep. The dry decomposed meat and fascia is nibbled from the bones and the sheep eat the softer bones of the carcass. The wool of the skin is usually left and one finds the wool lying scattered about on the veld.

The following conditions are noted in sheep of the Strandveld:—

1. Undeveloped bodies.
2. Reduced fertility of ewes.
3. Abnormally soft and light condition of the bones, resulting in frequent fractures, and a particular condition known as "Lamkruis," which is evidently brought about by fractures of the bones of the pelvis.
4. Osteophagia and allotriophagia.
5. A disease called "*Lamsiekte*."

When considering this group of conditions, the question naturally arises whether they result from a deficiency of phosphorus. That an aphosphorosis does exist in these animals is strongly suggested by the following facts:—

I. The soil and vegetation contain very little phosphorus as is evident from the results given in Table II.

II. The inorganic phosphorus content of the blood of sheep from this area is definitely below normal. The results of the blood analyses are given in Table III.

TABLE II.

Analysis of Soil and Vegetation from the Bredasdorp Area. Results given in percentages on the Dry Material.

Sample.	Moisture.	CaO.	Ca.	P_2O_5.	P.	No. of Analyses.
Soil.....	—	1·34	0·96	·09	·04	4
Grass.....	7·6	·88	·63	·27	·12	2
Shrubs.....	5·8	2·01	1·44	·115	·05	4

The vegetation consists mainly of shrubs which contain even less phosphorus than the grasses of that area. Lime is abundant in most parts of the district.

Table III.

Inorganic Phosphorus in the Blood of Sheep from the Bredasdorp District.

Sheep Grazed on the Strandveld for 14 Months.		Sheep Grazed on the Strandveld for 3 Months.	
Sheep No.	Inorg. P.	Sheep No.	Inorg. P.
	Inorganic Phosphorus in mg. per 100 c.c. blood.		
1	3·0	41	4·9
2	3·3	42	3·8
3	2·6	43	3·4
4	2·6	44	5·2
5	3·9	45	5·3
6	2·3	46	4·2
7	2·8	47	3·7
8	3·8	48	3·5
9	4·2	49	4·5
10	4·9	50	3·7
11	2·3	51	5·0
12	1·8	52	3·5
13	3·4	53	3·0
14	3·2	54	3·8
15	2·6	55	3·2
16	3·6	56	4·9
17	3·8	57	4·2
18	2·5	58	3·2
19	3·4	59	3·2
20	3·2	60	3·6
21	2·5	61	4·1
22	3·3	62	3·5
23	3·6	63	3·5
24	3·4	64	3·4
25	3·0	65	3·8
26	3·5	66	4·1
27	3·4	67	4·8
28	4·7	68	4·3
29	4·1	69	4·2
30	3·6	70	2·8
31	3·7	71	5·1
32	2·2	72	4·4
33	2·6	73	2·7
34	2·5	74	4·2
35	3·4	75	3·0
36	3·3	76	4·3
37	4·8	77	4·2
38	3·4	78	3·8
39	3·5	79	3·4
Average	3·3	Average	4·0

The above analyses were done at Vryburg three weeks after the sheep arrived there from Bredasdorp. The figure obtained for inorganic phosphorus is definitely below normal (±6.5 mg.) for both groups.

As a result of these findings one of the authors (S.D.R.) collected blood from sheep that were being kept on a typically Strandveld farm (Rietfontein). The average inorganic phosphorus content of the blood of 5 sheep was 2.4 mg. P per 100 c.c. blood. This figure is less than half that shown by animals on phosphorus sufficient pastures.

From the foregoing it seems practically certain that sheep grazing on the Strandveld of the Bredasdorp district do not get enough phosphorus for the demands of their bodies from the natural pasture. Calcium deficiency suggested by abnormal skeletal development is entirely negatived by the analysis of the soil and vegetation of the area in question. Furthermore, it seems very probable that lamsiekte in sheep in that area is associated with phosphorus deficiency in the same way as lamsiekte in cattle with aphosphorosis. The fact that carcass material which was actually being eaten by sheep, contained the organism responsible for lamsiekte in cattle further strengthens this view.

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Studies in Mineral Metabolism.—X.

THE EFFECT OF BONEMEAL FEEDING ON THE PHOSPHOROUS PARTITION OF THE BLOOD OF SHEEP.

By S. D. ROSSOUW, B.Sc., Research Officer, Onderstepoort.

Much work has been done on phosphorus metabolism by Green, Malan and other investigators, but their work has been confined, almost entirely, to cattle in conjunction with nutrition experiments at Armoedsvlakte. Accordingly an experiment on a small number of sheep was commenced on the 26th October, 1927. The nutritional part of the experiment was carried out as follows:—

Three sheep were kept on the basal ration only and were to serve as "controls" or "low phosphorus" animals. Three were given a bonemeal supplement of 10 grams per sheep per day, representing a "medium phosphorus" intake. Three other animals were placed on a "phosphorus high" diet, two, namely Nos. 16554 and 16362 received a bonemeal supplement of 30 grams and one (No. 15517) of 15 grams. One sheep (No. 15781) was kept on an approximately normal diet, i.e. the basal ration supplemented by 340 grams of green lucerne.

The basal ration consisted of 225 grams of teff hay (poor in phosphorus), 340 grams of flaked maize (Fanko) and 5 grams of salt. The sheep were dosed with the bonemeal every morning. The animals were kept in separate feeding boxes and allowed to exercise in a small camp from 9 a.m. to 3 p.m.

The phosphorus content of the flaked maize and teff hay used was very low. The phosphorus content of the basal ration only amounted to .36 grams of phosphorus per day.

The three groups represent approximately the following phosphorus intakes:—

Control animals ...	.36 grams per day.
Medium P.	1.36 grams per day.
High P.	1.86 grams per day and 3.36 grams per day.

Table 1.

Blood phosphorus before sheep entered into experiment.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
Controls.....	8529	20·7	10·5	10·2	4·8	5·4
„	14415	24·3	11·7	12·5	5·0	7·5
„	16145	19·2	10·2	9·0	4·5	4·5
Average.....	—	21·4	10·8	10·6	4·8	5·8
Medium Phosphorus	15651	21·7	10·9	10·7	4·9	5·8
„ „	15839	22·0	11·0	10·9	4·5	6·4
„ „	16184	22·2	10·7	11·1	5·0	6·1
Average.....	—	22·0	10·7	11·1	5·0	6·1
High Phosphorus..	15517	19·0	9·9	9·1	5·2	3·9
„ „ ..	16554	24·8	11·1	13·7	6·8	6·9
„ „ ..	16362	22·6	11·3	11·3	5·9	5·4
Average.....	—	22·1	10·8	11·4	6·0	5·4
Normal Diet.....	15781	22·8	11·8	11·0	4·6	6·4

Blood was drawn from the jugular vein and analysed for phosphorus according to the methods of H. H. Green (1928). The blood was first analysed a few days before the sheep entered the experiment. Until then they had been kept on the ordinary rations for sheep at this station. The results of the blood analysis appear in Table 1 and agree well with the normal figures for sheep in these parts.

At first the flaked maize appeared to be rather unpalatable, more than half the quantity given being refused, but after a few weeks the whole of the daily ration was consumed.

The next analysis (Table 2) was made two weeks after the experiment had started.

TABLE 2.

Blood Phosphorus, two weeks after sheep were placed on rations.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
Controls.....	8529	17·6	9·2	8·4	3·6	4·8
,, ...	14415	18·6	9·0	9·6	4·6	5·0
,, ...	16145	16·7	8·8	7·9	2·9	5·0
Average.....	—	17·6	9·0	8·6	3·7	4·9
Medium Phosphorus	15651	18·7	8·8	9·9	6·0	3·9
,, ,,	15839	18·0	9·1	8·9	4·0	4·9
,, ,,	16184	20·3	8·9	11·4	5·2	6·2
Average.....	—	19·0	8·9	10·1	5·1	5·0
High Phosphorus..	15517	18·9	9·9	9·0	4·5	4·5
,, ,, ..	16554	20·9	9·0	11·9	6·2	5·7
,, ,, ..	17362	21·4	10·1	11·3	6·2	5·1
Average.....	—	20·4	9·7	10·7	5·6	5·1
Normal Diet.....	15781	20·0	10·2	9·8	4·4	5·4

This shows a general drop in all the phosphorus fractions, which may possibly be accounted for by the sudden change of diet. The only factor that is rather striking is the inorganic phosphorus fraction, which shows a decided drop in the case of the controls. With all the other fractions this drop bears no relation to the phosphorus intake.

The next three tables represent the figures obtained by the blood analyses after four, seven and eleven weeks respectively.

TABLE 3.

Blood Phosphorus, four weeks after sheep were placed on rations.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
Controls.....	8529	18·1	8·7	9·4	3·8	5·6
,, ...	14415	18·1	9·4	8·6	3·4	5·2
,, ...	16145	16·6	8·8	7·2	2·1	5·1
Average.....	—	17·6	9·0	8·4	3·1	5·3
Medium Phosphorus	15651	20·9	9·0	10·9	5·6	5·3
,, ,,	15839	19·6	9·1	10·2	4·2	6·0
,, ,,	16184	18·2	9·0	9·2	4·5	4·7
Average.....	—	19·6	9·1	10·1	4·8	5·3
High Phosphorus..	15517	18·5	9·3	9·2	4·7	4·5
,, ,, ..	16554	19·8	8·3	11·5	5·7	5·8
,, ,, ..	16362	20·1	8·7	10·6	6·2	4·4
Average.....	—	19·5	8·8	10·4	5·5	4·9
Normal Diet.....	15781	19·3	10·1	8·2	4·0	4·2

TABLE 4.

Blood Phosphorus, seven weeks after sheep were placed on rations.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
Controls.....	8529	17·6	8·7	8·5	3·0	5·5
,, ...	14415	18·9	10·2	8·7	2·1	6·6
,, ...	16145	17·5	9·0	7·7	2·1	5·6
Average.....	—	18·0	9·3	8·3	2·4	5·9
Medium Phosphorus	15651	20·4	9·2	11·1	5·1	6·0
,, ,,	15839	19·3	8·8	9·6	4·4	5·2
,, ,,	16184	17·6	8·4	9·2	4·5	4·7
Average.....	—	19·1	8·8	10·0	4·7	5·3
High Phosphorus..	15517	17·4	9·0	8·4	4·0	4·4
,, ,, ..	16554	20·7	9·1	11·6	6·6	5·0
,, ,, ..	16362	21·4	9·5	11·9	5·9	6·0
Average.....	—	19·8	9·2	10·6	5·5	5·1
Normal Diet.....	15781	21·5	10·8	10·7	3·5	7·2

TABLE 5.

Blood Phosphorus, eleven weeks after sheep were placed on rations.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
Controls.....	8529	18·4	9·2	8·8	3·5	5·3
,, ...	14415	17·8	9·4	8·3	2·9	5·4
,, ...	16145	16·0	8·5	6·8	2·3	4·5
Average.....	—	17·4	9·0	8·0	2·9	5·1
Medium Phosphorus	15651	20·1	8·6	11·5	6·5	5·0
,, ,,	15839	19·3	9·4	9·4	4·7	4·7
,, ,,	16184	19·6	9·0	9·8	5·2	4·6
Average.....	—	19·7	9·0	10·2	5·5	4·8
High Phosphorus..	15517	19·4	9·0	9·6	5·6	4·0
,, ,, ..	16554	22·6	9·9	12·9	6·8	6·1
,, ,, ..	16362	21·8	9·6	12·4	6·6	5·8
Average.....	—	21·3	9·5	11·6	6·3	5·3
Normal Diet.....	15781	19·9	11·1	8·8	3·9	4·9

In Tables 3, 4 and 5 the inorganic phosphorus figures in the blood of the controls, show a further drop and reach an average figure as low as 2.9 mg. per 100 c.c. blood. With the " medium phosphorus " animals this fraction remains fairly constant, taken on an average, whereas in the case of the " high phosphorus " group there is a decided rise above the local normal value of 5.5 mg.

As in the case of bovines, the lipoid phosphorus seems to be unaffected by the addition of bonemeal to the ration (Tables 2, 3, 4 and 5). The same may be said of the organic acid soluble fraction, which remains practically constant throughout the experiment.

One week after the analyses noted in Table 5, the sheep were divided into two groups. The first group, the first five animals of Table 5, was given 30 grams of bonemeal. The other five received only the normal diet of teff, flaked maize and one pound of green lucerne from then onwards.

After a period of four months the blood was again analysed. The results are given in Table 6.

TABLE 6.

Group.	Sheep No.	Total P.	Lipoid P.	Total Acid Soluble P.	Inorganic P.	Organic Acid Soluble P.
High Phosphorus..	8529	20·5	9·4	11·2	6·4	4·8
,, ,, ..	14415	20·3	8·9	10·9	6·9	4·0
,, ,, ..	16145	19·7	8·8	10·3	7·0	3·3
,, ,, ..	15651	18·3	8·0	10·3	6·0	4·3
,, ,, ..	15839	18·4	8·4	10·3	6·0	4·3
Average.....	—	19·4	8·7	10·6	6·6	4·0
Normal Diet.....	16184	20·0	9·0	10·0	4·5	5·5
,, ,, ...	15517	17·4	9·3	8·2	4·0	4·2
,, ,, ...	16554	19·7	9·4	10·0	4·7	5·3
,, ,, ...	16362	17·3	8·7	8·6	4·5	4·1
,, ,, ...	15781	20·4	10·5	10·3	6·0	4·3
Average.....	—	18·9	9·4	9·4	4·7	4·7

Again, as will be noticed from the analysis of Table 6, the inorganic phosphorus clearly responded to the change in phosphorus intake, whereas no marked change is to be noticed in the figures of the lipoid and organic acid soluble phosphorus.

As all the sheep were full-grown wethers there was no change to be noticed in the weights during this short period of hardly three months, but, notwithstanding this, it was noticeable that the control animals were not in the same good condition as the others and that

the three " high phosphorus " animals were undoubtedly in a better condition. Sheep No. 16145 developed a weakness in the front legs towards the end of the period during which it was kept as a control, but on being placed on the high phosphorus diet it completely recovered.

Although this work must be considered as a preliminary study, it certainly points to important factors in relation to bonemeal or phosphorus feeding to sheep. A more elaborate experiment will be started in the near future.

The writer wishes to express his indebtedness to Dr. H. H. Green and Mr. Graf for valuable assistance and suggestions.

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Studies in Mineral Metabolism.—XI.

* MINERAL METABOLISM AND BLOOD ANALYSIS.

By A. I. MALAN, M.Sc., Biochemist, Onderstepoort.

Important and valuable data about mineral deficiencies in animals in South Africa have accumulated as a result of studying this problem chemically. Of the earlier investigators, Hutcheon, who began this work in 1884, must be mentioned. Other men have made valuable contributions in this field, as any of the publications on this problem will testify. H. H. Green continued the work in 1918 at Onderstepoort, when he undertook the analysis of pasture grasses for Phosphorus and Calcium from lamsiekte areas in connection with the study of lamsiekte or parabotulism in cattle. When it was realised that an adequate supply of Phosphorus was an essential factor in the prevention of this disease, as explained in the *Journal of the Department of Agriculture* of June, 1920, and secondly, that phosphorus feeding played an important rôle in the nutrition of stock over wide areas of the Union, the animal, instead of its feed, became the centre of study. Would a prime animal receiving an ample supply of phosphorus in its feed show differences in the mineral " make-up " of its body from that of a poor, apparently half-starved animal on a phosphorus deficient diet? This suggested the study of " Minimum Mineral Requirements of Cattle " by Theiler, Green, and Du Toit (1927). Detailed blood analyses were undertaken during the latter stages of that experiment, and the results definitely point to new means of investigation of the problem of mineral metabolism. It was also noticed that the outstanding characteristic of the blood of all the animals on a phosphorus low diet was its low inorganic phosphorus. Was this definitely diagnostic of aphosphorosis and might not deficiencies of other minerals be likewise reflected in the blood of animals? The investigation of these questions gave blood analysis a higher status in the study of mineral deficiencies. As a matter of fact, it now forms an integral part of all investigations at Onderstepoort into questions of mineral metabolism.

The work has been extended to include Sodium, Potassium, Magnesium, Chlorine, and lately Iodine, but its nucleus remains phosphorus and to a less extent Calcium metabolism. It may, therefore, not be out of place to include some general remarks on the determination of Phosphorus in the blood of animals in this paper, while referring readers to Green's paper (1928) on " The Determination of Phosphorus Compounds in the Blood by Dry Combustion " for a detailed statement of the method.

* Paper read to Section M of the British Association for the Advancement of Science at Pretoria in August, 1929.

The method for the determination of inorganic phosphorus is essentially the same as that of Bell and Doisy (1920), modified by Briggs (1924), which makes use of the colour reaction between phosphates and ammonium molybdate in presence of sulphuric acid with sodium sulphite and quinol as reducing agents. The determinations of total phosphorus, lipoid phosphorus, and of total acid soluble phosphorus are preceded by dry combustions for the destruction of organic matter as against the customary wet combustions. These are then followed by a modification of the ceruleo-molybdate method of Deníge (1920), where stannous chloride is used in acid solution as the reducing agent. A stable blue colour, whose intensity is proportional to the amount of phosphorus present, develops very rapidly and is matched against a standard in the colorimeter. The dry combustion methods have been tested against the wet methods, and found to be as accurate, more rapid, and more convenient for routine use. When workers have made themselves familiar with the usual precautions of microtechnique, the determination of the phosphorus partitions of blood by dry combustion is quite a simple matter and almost "foolproof." This is an important factor when large numbers of phosphorus partitions are made regularly and individual checking is therefore an impossibility. It may be said here that four phosphorus partitions, i.e. sixteen determinations, can be completed comfortably in an 8-hour working day, while with practice as many as eight partitions have been made.

One finds from the literature that most workers determine one less than the total number of phosphorus fractions in the blood and obtain the missing one by calculation. Although lipoid and total acid soluble phosphorus usually total up to the figure obtained for total phosphorus, there undoubtedly exists yet another small fraction in mammalian blood, viz., nucleoprotein, which would escape detection in this manner. In pathological cases where the blood erythrocytes are infested with nucleated organisms as in bovine piroplasmosis, or where a fair proportion of nucleated red cells are present, or where the blood leucocytes have increased as in certain stages of anaemia, this nucleoprotein is quite appreciable. In animals with nucleated erythrocytes like amphibians, reptiles, and birds, about fifty per cent. of the total phosphorus of the blood is present as nucleoprotein, as is shown in a publication by Malan in this report.

From the analysis of the blood of cattle on rations low in phosphorus and subsequently of the blood of cattle on phosphorus deficient pasture, it became evident that blood analysis could definitely be employed in the diagnosis of aphosphorosis.

Valuable and almost unlimited material for this work was found in the experiment on the breeding of cattle on phosphorus deficient pasture, reported by Theiler, Green, and Du Toit (1928). Two groups of cows under identical conditions of phosphorus deficient pasture, but with this difference, that the one received 5 oz. of bone-meal per head per day, whereas the other received none, provided the material for this investigation. The breeding experiment had been going on for three years, so that selections for blood analysis from both groups could be made to include animals of different ages, i.e. descendants of the original cows, and the results compared.

The greatest difference between the blood of the bonemeal batch and that of the control batch lay in the inorganic phosphorus fraction, although the total phosphorus showed a correspondingly lower figure for the control group than for the bonemeal group. The average inorganic phosphorus fraction of the blood of lactating cows on the bonemeal supplement was 3.1 mg. per 100 c.c. of whole blood and for the control cows only 1.5 mg. For dry cows the corresponding averages were 3.7 and 1.6 mg. The inorganic phosphorus fraction is thus approximately twice as high for the bonemeal lot as for the control lot, although 3.7 mg. is still below the average for cattle on a phosphorus rich pasture. For the heifers the difference was almost as striking, the corresponding figures being 5.2 and 2.5. mg. It should be said that, whereas the control lactating cows were obviously phosphorus deficient—clinical symptoms being noticeable—the heifers of the control group, although markedly inferior to the bonemeal ones, could not be said to be phosphorus deficient by one unacquainted with their history. Nevertheless, incipient styfsiekte or aphosphorosis could already be diagnosed from blood analysis.

If we examine the inorganic phosphorus fraction of the blood of the six months old heifer calves of the two lots of cows, there is only a tendency for the inorganic phosphorus to be lower for the blood of the control lot than for that of the bonemeal lot; the average figures are 4.6 and 3.4 mg. respectively. The calves of the control mothers were perhaps slightly inferior to those of the cows in the bonemeal group but a definite distinction could not be made. Yet, already the inorganic phosphorus fraction of the blood suggested phosphorus deficiency. In short, it may be said that aphosphorosis, even in the very early stages, is definitely reflected in the blood of cattle. Only in the later stages does such a condition become visible to the eye.

This opens up a host of possibilities for future work. A mineral survey may be made of phosphorus deficient areas in South Africa from blood analysis. Establishing the degree of phosphorus deficiency in the pasture and in a herd and studying the efficiency of various phosphatic supplements for the prevention of aphosphorosis are questions for the study of which blood analysis can be of inestimable value.

Although clinical aphosphorosis as such is practically unknown amongst sheep, a low inorganic phosphorus fraction (about 50 per cent. below normal) is shown in the blood of sheep from areas where cattle suffer from aphosphorosis. This indicates the necessity of investigating the question of phosphorus metabolism in the nutrition of sheep, and work in this direction is going on at Onderstepoort at present, while a series of determinations of phosphorus partitions in the blood is carried out simultaneously. Field work on this matter is comparatively easy; blood for analysis can be drawn, the proteins precipitated with trichloracetic acid, and the filtrates forwarded to Onderstepoort for inorganic phosphorus determination. Slight hydrolysis leading to an increase of inorganic phosphorus takes place. This matter has been dealt with in another article in this report.

In conclusion, blood analysis in relation to calcium metabolism may be mentioned. There is reason for believing that a low calcium-content of the feed of animals is reflected as low calcium in the blood.

The calcium determinations of the blood of a limited number of sheep (±100) from areas where the calcium-content of the soil and of the vegetation is very low, definitely show a figure which is below normal. Whether we are dealing with calcium deficiency in such cases is not yet known. Laboratory experiments with sheep and bovines on mineral metabolism, and lately on the part played by small quantities of iodine in nutrition, are now being conducted at Onderstepoort. A report of this work will be published in due course.

SUMMARY

1. An outline is given of a dry combustion method for the determination of phosphorus in blood.

2. Phosphorus deficiency or aphosphorosis produces a low inorganic phosphorus fraction in the blood.

3. This relation between aphosphorosis and low inorganic phosphorus in the blood has diagnostic value, and this is discussed.

4. Low blood calcium may be indicative of calcium deficiency.

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APPENDIX.

Since the above was written, facilities for carrying out a mineral survey of the Union have been granted, and this work is now in full progress. Numerous analyses of sheep blood for inorganic phosphorus in relation to phosphorus in the diet have been made, as is evident from the article by Du Toit, Malan, and Rossouw in this report. Some figures for inorganic phosphorus in the blood of sheep from phosphorus deficient areas are appended hereto, and illustrate the importance of regular blood analyses in connection with the study of aphosphorosis in sheep. One factor stands out clearly, viz., a low figure for inorganic phosphorus corresponding to a low phosphorus

intake in the diet. This apparently applies to sheep as it does to cattle. Occasionally individual analyses may show irregularity in the rise or fall of blood phosphorus, but this has little effect on the average figure for the group. In any case, no attempt will be made to gauge the degree of phosphorus deficiency in an area, or on a farm even, from the blood analysis of one animal. Table IV in the article by Du Toit, Malan, and Rossouw in this report offers an excellent opportunity for studying the relation between individual variations and the average figure for inorganic phosphorus.

INORGANIC PHOSPHORUS IN THE BLOOD OF SHEEP
Mg./100 c.c.

Nos.	Group I. Bonemeal Supplement.			Nos.	Group II. Salt Supplement.		
	May, 1929.	14/9/29.	20/9/29.		May, 1929.	14/9/29.	20/9/89.
25	3·0	6·1	6·0	73	2·7	3·6	2·9
26	3·5	6·5	6·7	74	4·2	2·9	3·1
27	3·4	7·0	6·5	75	3·0	4·5	4·7
28	4·7	5·7	5·1	76	4·3	3·4	2·9
29	4·1	6·3	6·7	77	4·2	3·7	3·5
30	3·6	6·8	7·3	78	3·8	3·2	3·4
31	3·7	5·9	5·6	79	3·4	4·0	3·2
32	2·2	5·2	6·2	13	3·4	3·6	3·4
33	2·6	7·7	6·8	14	3·2	3·4	2·7
34	2·5	4·3	5·5	15	2·6	3·4	3·9
Average	3·3	6·1	6·2		3·5	3·6	3·4

Nos.	Group III. Bonemeal and Salt Supplement.		Nos.	Group IV. Controls.		
	May, 1929.	14/9/29.		May, 1929.	14/9/29.	20/9/29.
1	3·0	6·3	13	3·4	3·6	3·4
2	3·0	—	14	3·2	3·4	2·7
3	2·6	6·5	15	2·6	3·4	3·9
4	2·6	5·0	16	3·6	3·8	3·5
5	3·9	5·4	17	3·8	4·0	3·4
6	2·3	6·1	18	2·5	2·8	2·1
7	2·8	5·8	21	2·5	2·8	2·6
8	3·8	5·0	22	3·3	3·2	2·9
9	4·2	5·7	23	3·6	3·8	2·9
10	4·9	7·6	24	3·4	4·0	3·9
Average	3·3	5·9		3·2	3·5	3·1

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

Studies in Mineral Metabolism.—XII.

PHOSPHORUS IN THE SHEEP INDUSTRY. PRELIMINARY REPORT.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services;
A. I. MALAN, M.Sc., Biochemist, Onderstepoort; and
S. D. ROUSSOUW, B.Sc., Research Officer, Onderstepoort.

Investigations which led to the publication of the series of articles "Studies in Mineral Metabolism" 1927 *et seq.* by Theiler, Green, Du Toit, and others, began with attempts to work out the etiology of the disease lamsiekte in cattle, afterwards shown to be a secondary result of phosphorus deficiency. Although the need for experiments with sheep in order to determine the rôle played there by phosphorus which was known to be deficient in South African pastures, was felt all along, numerical limitations of staff and the necessity of preventing huge annual losses of cattle through lamziekte demanded the concentration of all available hands on the study of phosphorus in relation to cattle farming, while experiments with sheep had to be postponed indefinitely. With the etiology of lamsiekte known, and various practical schemes of supplying phosphorus to cattle operating successfully, the prevention of losses in cattle other than incidental through lamsiekte may be said to be a matter of time only. Hence the time has come for a wider study of this problem of phosphorus deficiency in South Africa, and going further afield to the root of the problem where all classes of stock are concerned.

Much work has already been done in regard to incipient styfsiekte in cattle, but it cannot be said except from deductive reasoning in the light of the experiments with cattle, what rôle phosphorus plays in mutton and wool production.

Phosphorus feeding has been shown to be an essential factor in successful cattle farming. Even in areas where extreme deficiency, manifest as styfsiekte is not evident, the feeding of phosphatic supplements is more than paid for by the advantages such as better growth, fertility, increased milk yield, and the like. It seems, therefore, that phosphorus feeding to sheep, grazing over areas where cattle show various degrees of aphosphorosis, must be a factor that requires investigation. It was with a view to obtain more data on this side of the problem that the present work was undertaken. It must be said at the outset, however, that this report can be regarded only as preliminary, and that report will be made from time to time as the work progresses.

From the available literature, it seems that no thorough study has yet been made of the question of phosphorus in wool production. This is probably due to the fact that the evidence of phosphorus deficiency in sheep comparable with osteophagia and botulism in cattle is limited. Then, too, apparently an extremely low phosphorus intake satisfies the demands of the animals, or symptoms of phosphorus deficiency, such as stiffness, bone-chewing and general unthriftiness, do not become apparent in sheep as soon as in cattle. For instance, in some areas in Tasmania, according to Theiler and Orr (1929) where phosphorus deficiency " was present in an exaggerated measure in cattle, the sheep running on the same pasture were producing the finest wool." The skeletons of these sheep, on post mortem examination, showed signs of deficiency. It must be that sheep can either adapt themselves to remain apparently normal and healthy in areas where cattle would suffer from extreme phosphorus deficiency, or that the demands of the animals, being less than those in case of bovines, are satisfied on pastures too low in phosphorus-content for successful cattle farming. Probably both factors come into play. Extreme phosphorus deficiency or osteophagia in sheep is not unknown, however, and has been reported from various parts of the world. It appears in isolated areas, and although carrion poisoning of sheep (botulism) has been reported by Bekker (1930) in this country, and in Australia by Bennet (1928), it is by no means as common as in cattle. Areas are known in South Africa, especially along the coast, where cattle cannot be kept successfully on the natural pasture on account of phosphorus deficiency, and sheep remain stunted, very suceptible to disease, and will not breed unless removed to better pastures. These may be extreme cases of aphosphorosis in sheep, and at all events, if proved to be such, not difficult to diagnose; but even mild phosphorus deficiency can be studied very effectively from blood analysis. Malan (1930) showed that aphosphorosis even in the earliest stages may be diagnosed successfully by the determination of the inorganic phosphorus-content of the blood. In this, as in other work on deficiency diseases, regular blood analysis froms an integral part, and it has been applied to work on phosphorus deficiency with astounding success.

EXPERIMENTAL WORK.

An experiment was started at Onderstepoort in August, 1929, with the object of examining more accurately the value of phosphorus in the sheep industry. In brief outline the experiment is being conducted as follows:—Four groups of ten each of year-old ewes are kept under identical conditions in separate feeding boxes. The sheep are on the same basal ration, which is just above maintenance. The mineral composition of the ration differs only in its phosphorus-content, and in calcium in one sub-group, but further comment upon this will be left until later. Provision has been made for exercise, and by a system of rotation of sheep in the different boxes, external influences such as light and climate are equalized as nearly as possible. The exact food intake other than sodium phosphate and calcium carbonate, which are dosed and thus easily controlled, is registered daily, blood drawn weekly—lately fortnightly—for analysis for inorganic phosphorus, and the sheep weighed at first every month and

afterwards every fortnight. Records are kept of the individual sheep, and the experiment will be continued for a period of 2 or 3 years probably. The sheep have been selected by Government experts for uniformity of type and of wool.

The main object of the experiment is to note the effect of different quantities of phosphorus in the ration of sheep on wool production, growth, condition, food consumption, and fertility. In a general way the phosphorus intake is comparable with:

I. (Batch A) that in a ration as low as possible in phosphorus-content, the object being to produce clinical symptoms of phosphorus deficiency;

II. (Batch B) that of sheep on poor pasture which is abundant in phosphorus deficiency areas ($P_2O_5 = \cdot 25\ \%$);

III. (Batch C) that of sheep on excellent quality natural grass ($P_2O_5 = \cdot 5\ \%$);

IV. (Batch D) that greatly in excess of the demands of the animal body. (Equivalent to natural grass over 1 % P_2O_5.)

The calcium-content of the daily ration is 5 grams, which is equivalent to the calcium intake of sheep on natural grass of approximately ·80 % CaO. This applies to all the sheep, except half the number of Batch D, viz., Dii, which are on a low calcium ration with phosphorus content equivalent to that of Batch C. The sheep in Batch D, i.e. on excess phosphorus are under conditions not met with frequently in nature, hence it seemed that the material could be used more profitably by keeping only half the number under those conditions and noting for the remainder the effect of a low calcium ration with normal phosphorus on the conditions described above.

The daily basal ration consists of hay poor in minerals. This is gradually reduced to a minimum of 300 grams without causing digestive disturbances. The major food requirements are supplied by Fanko, which is rolled endosperm of maize and poor in minerals. Lysine and Tryptophane deficiency are obviated by adding 20 grams of bloodmeal. The mineral content of the ration, except phosphorus and calcium, is kept relatively constant, as is evident from a perusal of the rations given in Table I, by adding small quantities of two mineral mixtures. The protein-content of the ration is based on standard requirements according to Henry and Morrison.

The general scheme of the experiment, and the total daily intake of the animals, are given in Table I.

TABLE I.—GENERAL SCHEME OF EXPERIMENT.

Batch.	Description.	Ration.	Dry Matter. Grass.	Crude Protein.	Ca.
	Extreme phosphorus deficiency	Hay	275·0	19·8	0·78
		Fanko	430·0	35·5	0·03
A.		Bloodmeal (Basal ration)	20·0	12·9	0·03
		Mineral mixture A (Basal ration)	3·5	—	—
10 Sheep		Total	728·5	68·2	0·84
		NaCl	8·0	—	—
		$CaCO_3$	9·5	—	3·81
		Tap water 31 litres	—	—	0·30
		Total	746·0	68·2	4·95
Milligram	equivalents		—	—	247
	Ration low in phosphorus	Basal ration	728·5	68·2	0·84
		NaCl	7·0	—	—
B.		$CaCO_3$	9·5	—	3·81
		Na_2HPO_4	2·0	—	—
10 Sheep		Tap water 31 litres	—	—	0·30
		Total	747·0	68·2	4·95
Milligram	equivalents		—	—	247
	Phosphorus sufficiency	Basal ration	728·5	68·2	0·84
		NaCl	4·0	—	—
C.		$NaCO_3$	9·5	—	3·81
		Na_2HPO_4	8·0	—	—
10 Sheep		Tap water 31 litres	—	—	0·30
		Total	750·0	68·2	4·95
Milligram	equivalents		—	—	247
	Excess phosphorus	Basal ration	728·5	68·2	0·84
Di.		$CaCl_2$	7·5	—	2·70
		$CaCO_3$	2·5	—	1·00
5 Sheep		Na_2HPO_4	19·5	—	—
		Tap water 31 litres	—	—	0·30
		Total	757·0	68·2	4·84
Milligram	equivalents		—	—	242
	Calcium deficiency and phosphorus sufficiency	Hay	275·0	19·8	0·78
		Fanko	430·0	35·5	0·03
Dii		Blood meal	20·0	12·9	0·03
		Mineral mixture B	9·0	—	—
5 Sheep		Na_2HPO_4	8·0	—	—
		Rain water	—	—	—
		Total	742·0	68·2	0·84
Milligram	equivalents		—	—	42

COMPOSITION OF DAILY RATION.

P.	Na.	K.	Mg.	Fe.	NH_3.	Cl.	SO_3.	Citrate.	Energy Therms.
0·23	0·03	2·96	0·54	—	—	0·60	1·29	—	2·5
0·17	0·05	5·35	0·11	—	—	0·13	0·6	—	25·5
0·07	0·21	0·06	0·2	—	—	0·07	0·15	—	0·03
—	—	0·79	0·07	0·19	0·05	0·71	1·38	1·11	—
0·47	0·29	9·16	0·74	0·19	0·05	1·51	3·42	1·11	28·03
—	3·15	—	—	—	—	4·85	—	—	—
—	—	—	—	—	—	—	—	—	—
—	—	—	0·25	—	—	—	—	—	—
0·47	3·44	9·16	0·99	0·19	0·05	6·36	3·42	1·11	28·0
46	150	235	82	10	3	179	86	17	—
0·47	0·29	9·16	0·74	0·19	0·05	1·51	3·42	1·11	28·0
—	2·76	—	—	—	—	4·24	—	—	—
—	—	—	—	—	—	—	—	—	—
0·26	0·39	—	—	—	—	—	—	—	—
—	—	—	0·25	—	—	—	—	—	—
	3·44								
0·73		9·16	0·99	0·19	0·05	5·75	3·42	1·11	28·0
71	150	235	82	10	3	162	86	17	—
0·47	0·29	9·16	0·74	0·19	0·05	1·51	3·42	1·11	28·0
—	1·58	—	—	—	—	2·42	—	—	—
—	—	—	—	—	—	—	—	—	—
1·06	1·57	—	—	—	—	—	—	—	—
—	—	—	0·25	—	—	—	—	—	—
1·53	3·44	9·16	0·99	0·19	0·05	3·93	3·42	1·11	28·0
148	150	235	82	10	3	111	86	17	—
0·47	0·29	9·16	0·74	0·19	0·05	1·51	3·42	1·11	28·0
—	—	—	—	—	—	4·80	—	—	—
—	—	—	—	—	—	—	—	—	—
2·45	3·65	—	—	—	—	—	—	—	—
—	—	—	0·25	—	—	—	—	—	—
2·92	3·94	9·16	0·99	0·19	0·05	6·31	3·42	1·11	28·0
283	171	235	82	10	3	178	86	17	—
0·23	0·03	2·96	0·54	—	—	0·60	1·29	—	2·5
0·17	0·05	5·35	0·11	—	—	0·13	0·60	—	25·5
0·07	0·21	0·06	0·02	—	—	0·07	0·15	—	0·3
—	1·49	1·17	0·11	0·29	0·08	3·37	1·98	1·67	—
1·06	1·57	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—
1·53	3·35	9·54	0·78	0·29	0·08	4·17	4·02	1·67	28·0
148	144	244	65	16	4	117	100	27	—

TABLE II.—WEIGHTS OF SHEEP IN KG.

D.O.B. Nos.	Preliminary Period.				Experimental Period.						Increase.
	12.8.29.	30.8.29.	16.9.29.	2.10.29.	31.10.29.	14.11.29.	30.11.29.	15.12.29.	30.12.29.	14.1.30.	
Batch A. Extreme Phosphorus Deficiency.											
23964	32·2	29·5	Fleeces	27·7	27·7	27·6	27·2	27·1	27·2	26·3	— 1·4
23965	39·5	35·4	Clipped.	31·8	33·6	33·4	33·1	34·0	34·0	32·6	1·8
24005	35·0	34·8	,,	32·7	32·6	32·2	33·6	33·6	33·6	32·7	Nil
23967	32·7	31·5	,,	29·1	29·1	28·7	28·6	28·7	29·1	28·1	— 0·1
23968	33·6	32·4	,,	30·0	29·5	29·5	30·0	32·2	31·8	30·4	0·4
23969	39·5	36·8	,,	34·5	35·4	35·0	36·3	36·8	37·7	36·8	2·3
23970	32·7	30·6	,,	28·1	27·2	26·3	27·5	27·7	28·1	27·2	— 0·9
23971	30·4	25·6	,,	23·6	25·0	24·0	24·5	24·0	24·5	23·6	Nil
23972	29·5	28·5	,,	26·3	26·0	26·8	25·9	24·5	25·4	24·5	— 1·8
23973	33·0	31·5	,,	29·1	26·3	25·6	25·0	25·4	25·4	28·1	— 1·0
Averages	33·8	31·7	,,	29·3	29·2	28·9	29·2	29·4	29·7	29·1	— 0·2
Batch B. Low Phosphorus.											
23974	30·9	28·1	,,	25·0	24·0	25·0	27·2	28·6	29·5	29·5	4·5
23975	35·4	35·0	,,	32·7	34·0	34·5	35·0	35·4	37·2	36·3	3·6
23976	33·6	30·6	,,	28·6	28·6	29·5	30·4	30·9	32·7	34·0	5·4
23977	31·8	30·0	,,	27·7	29·5	29·7	29·1	30·4	31·4	30·4	2·7
23978	36·8	35·0	,,	32·7	34·0	34·0	35·4	36·3	37·2	36·8	4·1
23979	33·6	31·8	,,	30·0	30·9	30·0	31·4	31·4	31·4	32·7	2·7
23980	32·2	29·5	,,	26·3	26·8	26·4	27·2	27·7	29·1	28·6	2·3
23981	32·7	31·5	,,	29·1	30·4	29·5	30·4	32·2	33·1	31·8	2·7
23982	34·5	32·5	,,	30·1	30·4	30·4	31·4	31·4	32·2	31·8	1·7
23983	32·7	35·0	—	32·5	30·4	30·0	30·4	31·8	32·2	31·8	— ·7
Averages	33·4	31·9	—	29·7	29·9	29·9	30·8	31·6	32·6	32·4	2·9

TABLE II.—WEIGHTS OF SHEEP IN KG.—(*continued*).

D.O.B. Nos.	Preliminary Period.				Experimental Period.						Increase.
	12.8.29.	30.8.29.	16.9.29.	2.10.29.	31.10.29.	14.11.29.	30.11.29.	15.12.29.	30.12.29.	14.1.30.	
Batch C. Phosphorus Sufficiency.											
23984	35·0	33·0	Fleeces	30·9	31·8	32·7	32·7	34·0	35·0	34·5	3·6
23985	35·4	32·2	Clipped.	30·4	31·4	30·9	30·9	31·4	33·1	32·7	2·3
23986	37·2	35·0	,,	32·7	32·0	32·7	33·6	34·5	36·8	36·3	3·6
23987	31·4	30·0	,,	27·7	27·7	27·7	29·5	31·4	30·9	30·9	3·2
23988	35·9	35·0	,,	32·2	33·6	33·6	35·0	36·3	36·8	37·7	5·5
23989	34·5	30·9	,,	27·7	30·9	30·4	29·5	30·0	31·8	31·8	4·1
23990	32·7	31·5	,,	29·1	31·4	30·0	31·4	31·8	32·2	32·2	3·1
23991	35·0	33·2	,,	30·9	31·8	29·5	30·0	31·4	33·1	34·0	3·1
23992	36·3	35·6	,,	33·1	32·7	32·7	33·1	34·0	35·9	35·9	2·8
23993	34·0	31·4	,,	27·7	30·9	31·4	32·2	33·6	35·0	34·5	6·8
Averages	34·74	32·78	,,	30·24	31·42	33·16	31·79	32·84	34·06	34·05	3·8
Batch Di. Excess Phosphorus.											
23994	31·4	31·0	,,	27·7	28·0	27·2	28·6	29·8	30·9	30·4	2·7
23995	32·7	30·6	,,	28·1	29·0	28·6	29·5	29·8	31·4	31·4	3·3
23996	36·3	32·8	,,	30·4	29·5	26·8	27·2	30·0	32·2	31·8	1·4
23997	36·3	35·0	,,	32·7	33·1	32·7	32·0	31·4	31·8	34·0	1·3
23998	35·4	34·0	,,	30·9	33·1	32·7	31·8	32·2	31·8	31·4	0·5
Averages	34·4	32·68	,,	29·96	30·54	29·6	29·8	30·5	31·6	31·8	1·8
Batch Dii. Calcium Deficiency. Phosphorus Sufficiency.											
23999	36·3	34·0	,,	31·8	32·0	32·2	33·1	34·0	37·2	36·3	4·5
24000	32·2	31·0	,,	28·6	30·4	30·4	30·4	32·7	34·0	34·0	5·4
24001	35·0	32·8	,,	30·4	31·4	31·8	32·2	32·7	34·5	34·0	3·6
24002	34·0	33·2	,,	30·9	30·4	30·0	29·1	30·4	31·4	30·9	Nil.
24003	28·6	26·8	,,	24·5	25·4	25·0	25·9	27·2	29·5	28·6	4·1
Averages	33·2	31·56	,,	29·2	29·9	29·9	30·14	31·4	33·3	32·76	3·5

TABLE III.—FOOD CONSUMPTION. RATIONS IN KG.; ENERGY IN THERMS

D.O.B. Numbers.	Preliminary Period.				Averages.		Total Nett Energy of Averages.	Experimental Period.						Averages.		Total Nett Energy of Averages.
	Aug. 8–Sept. 7.		Sept. 8–Oct. 7.					Oct. 8–Nov. 7.		Nov. 8–Dec. 7.		Dec. 8–Jan. 7.				
	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.		Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	Hay.	Fanko.	
Batch A. Extreme Phosphorus Deficiency.																
23964.....	5·6	8·7	5·8	10·7	5·7	9·7	23·1	5·6	11·5	5·4	9·7	3·6	8·5	4·8	9·9	23·1
23965.....	6·9	10·2	7·6	9·6	7·25	9·9	24·3	8·5	11·6	7·4	11·4	5·6	12·3	7·0	11·8	28·1
24006.....	—	—	—	—	—	—	—	7·2	8·2	5·3	9·7	3·8	8·7	5·4	8·9	21·2
23967.....	5·6	9·5	6·7	10·8	6·15	10·15	24·0	5·6	11·7	3·7	9·5	2·9	7·6	4·1	9·6	22·1
23968.....	3·6	10·3	4·3	11·4	3·95	10·8	24·4	3·5	11·1	3·8	9·6	2·9	8·9	3·4	9·9	22·4
23969.....	3·5	10·4	5·7	11·9	4·6	11·15	25·5	5·1	13·0	5·0	11·7	3·6	12·2	4·6	12·3	28·0
23970.....	5·7	7·9	5·5	10·1	5·6	9·0	21·5	4·0	10·8	3·4	9·3	4·0	9·9	3·8	10·0	22·8
23971.....	2·9	6·7	4·5	8·0	3·7	7·35	17·1	4·9	8·0	3·8	8·7	3·5	8·8	4·1	8·5	19·8
23972.....	4·4	11·4	4·8	10·0	4·6	10·7	24·6	4·1	8·0	4·5	5·1	4·1	5·6	4·6	6·2	15·3
23973.....	6·1	10·0	6·0	8·9	6·05	9·45	22·6	6·0	6·0	5·0	12·9	5·0	7·2	5·3	8·7	20·8
Averages.....	4·9	9·45	5·6	10·2	—	—	23·1	5·45	10·0	4·7	9·8	3·9	8·9	—	—	22·3
Energy.....	2·9	19·8	2·7	21·3	—	—	—	2·6	20·9	2·3	20·5	1·9	18·6	—	—	—
TOTALS....	22·2		24·05					23·5		22·8		20·5				
Batch B. Phosphorus Deficiency.																
23974.....	6·3	9·2	8·0	7·2	7·2	8·2	20·6	7·1	8·0	6·3	11·3	6·5	11·7	6·6	10·3	24·7
23975.....	5·5	11·3	6·9	11·7	6·2	11·5	27·0	6·8	12·8	5·9	12·7	5·1	12·8	5·9	12·7	29·5
23976.....	5·4	10·0	6·7	10·6	6·1	10·3	24·5	5·9	11·1	5·4	11·5	6·8	11·9	6·0	11·5	26·9
23977.....	4·3	11·7	5·2	10·8	4·7	11·2	25·7	5·4	11·4	6·2	12·0	7·8	12·3	6·4	11·9	27·9
23978.....	5·7	11·9	7·0	12·2	6·4	12·1	25·1	7·5	12·3	7·6	12·9	8·0	13·0	7·7	12·7	30·3
24005.....	6·1	10·9	7·3	10·9	6·7	10·9	25·0	7·0	12·3	6·1	11·7	5·4	12·0	6·1	12·0	28·1
23980.....	5·9	9·6	6·9	9·5	6·4	9·5	22·9	7·0	9·9	6·5	9·8	6·4	9·5	6·6	9·7	23·5
23981.....	4·4	12·4	5·7	9·2	5·0	10·8	25·0	6·0	11·8	6·5	11·0	5·9	11·4	6·1	11·4	26·7
23982.....	6·6	10·0	6·2	11·2	6·4	10·6	25·2	6·8	10·2	6·7	8·7	6·1	8·9	6·6	9·3	22·7
23983.....	5·4	10·7	6·4	10·8	5·9	10·7	25·2	6·8	11·9	6·5	11·7	6·1	11·0	6·5	11·5	27·2
Averages.....	5·6	10·8	6·6	10·4	—	—	25·1	6·6	11·2	6·4	11·3	6·5	11·4	—	—	26·8
Energy.....	2·7	22·6	3·2	21·7	—	—	—	3·2	23·4	3·1	23·6	3·2	23·8	—	—	—
TOTALS....	25·3		24·9					26·6		26·7		27·0				

TABLE III.—FOOD CONSUMPTION. RATIONS IN KG.; ENERGY IN THERMS—*(Continued)*.

D.O.B. Numbers.	Preliminary Period. Aug. 8–Sept. 7. Hay.	Aug. 8–Sept. 7. Fanko.	Sept. 8–Oct. 7. Hay.	Sept. 8–Oct. 7. Fanko.	Averages. Hay.	Averages. Fanko.	Total Nett Energy of Averages.	Experimental Period. Oct. 8–Nov. 7. Hay.	Oct. 8–Nov. 7. Fanko.	Nov. 8–Dec. 7. Hay.	Nov. 8–Dec. 7. Fanko.	Dec. 8–Jan. 7. Hay.	Dec. 8–Jan. 7. Fanko.	Averages. Hay.	Averages. Fanko.	Total Nett Energy of Averages.
Batch C. Phosphorus Sufficiency.																
23984.....	5·8	10·3	6·4	10·1	6·1	10·2	24·3	8·3	11·2	7·3	11·1	8·0	11·1	7·83	11·13	27·0
23985.....	5·1	11·3	6·0	10·8	5·55	11·05	25·8	5·6	11·0	6·8	8·9	6·5	10·7	6·3	10·2	24·4
23986.....	4·2	12·1	6·8	10·0	5·5	11·05	25·6	6·0	11·4	6·3	12·1	5·8	12·4	6·0	11·96	27·9
23987.....	5·0	9·3	6·5	9·4	5·75	9·35	23·3	7·1	10·3	6·6	12·0	7·2	10·3	6·96	10·86	26·1
23988.....	6·1	10·8	6·5	10·4	6·3	10·6	25·4	7·9	13·0	7·6	12·3	7·2	13·1	7·56	12·8	30·5
23989.....	8·0	7·3	7·7	10·4	7·85	8·8	22·2	7·1	12·5	5·9	11·5	5·8	11·4	6·26	11·8	27·8
23990.....	6·1	11·8	6·7	11·8	6·4	11·8	27·9	6·7	12·0	6·7	10·5	6·5	11·8	6·63	11·4	27·0
23991.....	7·0	9·7	7·6	9·4	7·3	9·55	23·6	6·7	10·3	6·0	10·2	7·8	11·5	6·8	10·6	25·5
23992.....	5·1	12·1	5·4	12·1	5·25	12·1	27·9	5·9	11·7	5·3	11·7	5·3	11·7	5·5	12·0	27·8
23993.....	5·4	11·1	6·1	10·7	5·75	10·9	25·6	6·1	12 1	6·9	12·9	6·4	12·7	6·46	12·56	29·5
Averages.....	5·78	10·58	6·57	10·6	—	—	24·9	6·74	10·55	6·54	11·32	6·65	11·72	—	—	26·7
Energy.....	2·6	22·1	3·0	22·1	—	—	—	3·3	22·1	3·4	23·7	3·4	24·5	—	—	—
TOTALS....	24·7		25·1					25·1		27·0		2·78				
Batch Di. Excess Phosphorus.																
23994.....	5·2	9·3	7·0	10·6	6·1	9·9	23·7	7·0	10·1	6·5	9·2	6·9	10·2	6·8	9·83	23·9
23995.....	4·6	9·8	5·8	10·6	5·2	10·2	23·8	5·0	11·2	5·7	9·7	5·4	11·2	5·36	10·7	25·0
23996.....	7·7	7·6	8·1	9·6	7·9	8·6	21·8	7·4	9·7	6·3	8·6	5·4	11·1	6·36	9·8	23·6
23997.....	5·5	11·4	6·5	11·8	6·0	11·6	27·1	6·8	12·5	5·9	12·3	4·8	11·8	5·83	12·2	28·3
23998.....	4·6	7·4	6·5	10·5	5·56	8·9	21·3	6·9	10·9	7·0	11·2	6·7	10·3	6·86	10·8	25·9
Averages.....	5·5	9·1	6·8	10·6	—	—	23·6	6·6	10·9	6·3	10·2	5·8	10·9	—	—	25·3
Energy.....	2·7	19·0	3·3	22·2	—	—	—	3·2	22·8	3·1	21·3	2·8	22·8	—	—	—
TOTALS....	21·7		25·5					2·60		24·4		25·6				
Batch Dii. Phosphorus Sufficiency. Calcium Deficiency.																
23999.....	4·9	12·3	5·9	10·9	5·4	11·6	26·8	5·3	12·4	5·8	12·4	6·6	12·5	5·9	12·43	28·8
24000.....	4·7	10·5	5·6	10·7	5·15	10·6	24·6	5·8	12·3	5·7	11·7	5·3	12·0	5·6	12·0	27·8
24001.....	5·1	10·8	6·2	11·4	5·65	11·1	26·0	6·0	11·6	6·0	10·1	6·6	11·4	6·2	11·03	26·0
24002.....	5·1	11·7	6·0	11·9	5·55	11·8	27·4	6·1	11·2	6·0	6·5	5·4	9·7	5·83	9·16	21·9
24003.....	4·6	6·7	4·7	8·0	4·65	7·35	17·5	4·6	9·3	4·9	9·8	5·5	11·3	5·0	10·13	23·6
Averages.....	4·9	10·4	5·7	10·6	—	—	24·5	6·09	11·13	5·92	10·15	5·86	11·15	—	—	25·4
Energy.....	2·4	21·7	2·8	22·2	—	—	—	3·0	23·2	2·9	21·1	2·9	23·2	—	—	—
TOTALS....	24·1		25·0					26·2		24·0		26·1				

TABLE IV.—INORGANIC PHOSPHORUS AND CALCIUM CONTENT OF BLOOD IN MGM./100 C.C.

Batch A. Extreme Phosphorus Deficiency.

D.O.B. Nos.	Preliminary Period.									Experimental Period.																		
	12/8/29.	19/8.	26/8.	2/9.	9/9.	16/9.	23/9.	30/9.	3/10.	14/10.	21/10.	28/10.	4/11.	11/11.	18/11.	25/11.		2/12.		16/12.		30/12.		14/1/30.				
																	Ca.		Ca.		Ca.		Ca.		Ca.		Ca.	
23964	8·7	5·4	6·4	3·6	3·8	—	5·1	5·0	—	3·6	3·9	3·9	4·2	2·9	2·4	2·9	7·6	2·2	7·9	2·5	8·4	2·6	8·3	2·2	9·2			
23965	6·8	5·2	4·5	5·0	4·5	—	5·4	5·3	—	4·2	4·3	4·0	3·4	3·5	2·9	3·0	7·6	1·9	8·0	2·3	8·6	2·7	8·0	1·8	7·8			
24004	5·6	4·7	6·8	6·1	5·5	—	—	5·3	—	5·9	4·1	3·4	2·0	2·3	2·0	1·6	8·0	1·9	8·5	1·5	9·2	1·7	8·0	1·9	8·8			
23967	10·7	5·9	7·6	5·6	4·3	—	5·1	3·7	—	3·4	2·8	3·2	2·7	2·4	2·6	2·8	8·5	2·1	8·4	2·2	8·5	2·1	7·8	2·1	7·6			
23968	5·5	5·4	4·9	4·7	4·1	—	5·0	5·0	—	3·5	4·0	3·9	2·7	3·2	3·0	2·8	8·6	2·3	8·6	1·9	9·1	2·6	7·8	2·0	8·4			
23969	8·0	5·2	4·4	4·3	4·0	—	5·0	5·8	—	4·5	3·9	3·9	2·8	3·3	2·7	3·0	—	1·7	—	2·2	—	2·1	—	1·8	—			
23970	9·0	5·8	6·2	3·7	3·9	—	4·3	3·9	—	3·5	3·7	3·2	3·0	3·2	3·1	3·0	—	2·3	—	2·7	—	2·6	—	2·1	—			
23971	6·7	4·7	7·9	5·2	5·0	—	4·1	5·6	—	—	4·6	4·3	4·1	3·6	2·6	2·1	—	2·1	—	2·2	—	2·6	—	2·1	—			
23972	7·1	6·6	6·4	4·2	7·0	—	5·8	4·1	—	3·8	3·4	—	2·5	3·4	2·9	4·2	—	2·2	—	2·5	—	—	—	1·9	—			
23973	5·5	4·5	3·9	3·8	4·8	—	6·4	6·2	—	5·1	7·0	7·1	6·6	3·9	4·9	6·6	—	4·3	—	2·3	—	1·7	—	3·7	—			
Averages	7·4	5·3	5·9	4·5	4·7	—	5·1	5·1	—	4·5	4·7	4·1	3·4	3·2	2·9	3·2	8·1	2·3	8·3	2·2	8·4	2·3	8·0	2·2	8·3			

Batch B. Low Phosphorus.

23974	5·3	6·0	7·5	4·4	5·3	—	7·3	5·0	—	4·9	3·9	3·2	4·9	3·9	5·5	6·0	—	4·6	—	5·7	—	5·4	—	3·9	—			
23975	5·2	5·5	5·8	4·4	4·1	—	5·7	5·4	—	5·9	5·7	5·4	4·7	4·2	5·0	6·2	—	4·2	—	4·8	—	4·9	—	3·1	—			
23976	—	5·0	6·6	4·8	5·5	—	5·4	4·2	—	4·4	4·3	3·4	5·2	3·7	3·1	3·6	—	3·0	—	4·0	—	3·8	—	3·9	—			
23977	5·6	4·8	6·1	4·6	—	—	6·1	5·3	—	4·5	5·2	6·2	4·2	4·6	4·8	4·5	—	4·0	—	4·5	—	4·9	—	4·2	—			
23978	6·5	5·9	7·5	5·0	4·7	—	6·1	5·9	—	5·1	5·1	5·6	5·7	5·2	5·6	4·6	—	5·1	—	4·8	—	5·9	—	4·4	—			
24005	5·9	5·6	5·8	4·8	5·5	—	6·0	4·7	—	4·4	5·6	4·3	4·3	3·7	3·2	3·7	—	2·9	—	3·6	—	4·0	—	3·6	—			
23980	4·1	4·5	7·0	6·3	4·6	—	4·7	4·8	—	5·3	3·9	4·2	3·6	3·4	3·8	4·3	—	4·2	—	5·2	—	4·2	—	4·0	—			
23981	6·7	—	6·1	5·5	—	—	4·5	5·8	—	4·1	3·7	4·5	4·7	4·5	3·3	4·4	—	4·7	—	5·3	—	4·5	—	3·5	—			
23982	8·3	4·7	6·8	4·5	—	—	4·8	4·8	—	5·1	5·1	4·2	4·9	4·7	4·4	4·6	—	3·4	—	4·1	—	4·2	—	3·4	—			
23983	7·7	5·4	5·8	6·0	5·9	—	5·9	6·0	—	6·5	6·7	—	4·3	5·5	4·8	5·2	—	5·5	—	5·6	—	5·0	—	3·5	—			
Averages	6·1	5·3	6·5	5·0	5·1	—	5·6	5·2	—	5·0	4·9	4·5	4·6	4·3	4·4	4·7	—	4·2	—	4·8	—	4·7	—	3·8	—			

TABLE IV.—INORGANIC PHOSPHORUS AND CALCIUM CONTENT OF BLOOD IN MGM./100 C.C.—(*Continued*).

Batch C. Phosphorus Sufficiency.

D.O.B. Nos.	Preliminary Period.									Experimental Period.																
	12/8/29.	19/8.	26/8.	2/9.	9/9.	16/9.	23/9.	30/9.	3/10.	14/10.	21/10.	28/10.	4/11.	11/11.	18/11.	25/11.	2/12.		16/12.		30/12.		14/1/30.			
																	Ca		Ca.		Ca.		Ca.		Ca.	
23984	8·5	6·5	4·9	6·1	6·4	—	—	5·9	—	7·5	10·0	6·4	8·4	5·8	8·1	6·8	—	6·2	—	7·0	—	8·2	—	5·4	—	
23985	5·2	6·0	6·5	4·6	4·0	—	6·2	4·3	—	6·0	5·7	7·3	6·4	9·0	5·2	7·5	—	4·8	—	6·0	—	6·0	—	6·2	—	
23986	7·3	6·3	—	5·7	—	—	4·3	5·9	—	9·1	9·9	8·6	7·1	8·3	8·8	9·5	—	7·5	—	7·7	—	7·4	—	6·0	—	
23987	6·9	8·6	8·2	7·2	8·6	—	7·1	6·4	—	7·2	—	7·1	9·2	8·5	8·1	9·1	—	6·1	—	8·8	—	8·2	—	7·8	—	
23988	9·4	4·8	5·1	4·7	5·3	—	6·1	5·6	—	6·7	7·9	6·9	6·9	6·9	7·2	7·4	—	6·6	—	6·9	—	6·4	—	6·2	—	
23989	5·5	6·8	5·4	4·9	5·1	—	6·3	6·7	—	5·4	6·7	6·1	6·5	7·4	5·7	5·6	—	6·2	—	6·7	—	6·6	—	5·7	—	
23990	8·8	8·1	4·9	4·8	4·7	—	5·9	5·5	—	9·1	10·8	10·2	8·4	9·1	10·7	10·9	—	6·6	—	7·5	—	7·0	—	6·9	—	
23991	7·6	6·5	9·7	7·0	7·8	—	6·4	6·7	—	10·0	9·2	8·0	6·7	7·8	9·8	7·8	—	6·7	—	8·5	—	9·1	—	8·7	—	
23992	9·8	5·4	6·3	4·7	4·4	—	4·4	4·4	—	6·6	9·4	7·4	6·6	6·8	7·1	6·9	—	7·5	—	6·5	—	7·0	—	6·2	—	
23993	3·3	3·9	4·5	4·1	4·4	—	5·1	5·3	—	5·8	6·7	6·5	6·5	5·1	7·2	6·2	—	5·6	—	6·5	—	6·4	—	6·0	—	
Averages	7·2	6·3	6·1	5·4	5·7	—	5·9	5·7	—	7·3	8·4	7·4	7·2	7·4	7·8	7·8	—	6·4	—	7·2	—	7·2	—	6·5	—	

Batch Di. Excess Phosphorus.

13994	7·3	5·8	6·5	4·9	6·2	—	5·6	4·8	—	9·6	10·3	7·5	7·8	8·2	6·5	6·1	8·7	6·6	9·0	8·0	8·5	9·8	7·4	7·0	—	
23995	5·2	7·6	4·9	3·5	3·8	—	5·6	6·0	—	8·2	8·7	6·6	6·5	7·9	7·5	9·1	8·1	5·8	9·2	7·5	8·5	7·2	6·0	5·2	—	
23996	8·3	6·8	5·1	6·2	7·5	—	7·4	5·5	—	7·4	7·0	8·3	8·2	7·9	10·6	5·2	8·5	5·0	—	6·1	7·0	6·2	6·6	4·7	—	
23997	6·1	5·0	4·0	3·7	4·4	—	5·7	5·6	—	7·3	6·4	8·1	6·5	4·5	7·0	5·6	8·6	7·9	—	5·5	8·0	6·6	6·5	6·6	—	
23998	8·0	5·4	5·8	5·1	8·6	—	5·8	5·0	—	6·1	6·3	8·6	5·7	8·2	8·6	8·9	8·3	5·4	7·8	7·1	7·6	6·0	8·1	6·4	—	
Averages	7·0	6·1	5·3	5·1	6·1	—	6·0	5·4	—	7·7	7·7	7·8	6·9	7·3	8·0	6·9	8·4	6·1	8·7	6·8	7·9	7·2	6·9	6·0	—	

Batch Dii. Calcium Deficiency. Phosphorus Sufficiency.

23999	4·5	7·0	6·1	5·0	5·8	—	6·4	5·4	—	7·4	7·8	7·2	7·4	8·0	9·5	8·4	8·8	7·5	7·5	7·7	8·4	6·6	5·8	6·7	7·0	
24000	5·1	5·5	4·4	5·5	5·0	—	5·9	4·1	—	5·6	6·4	6·1	4·4	5·8	6·0	7·5	8·6	7·4	—	5·4	8·4	5·1	6·6	4·6	7·6	
24001	6·4	5·8	5·6	5·1	4·0	—	6·5	3·8	—	5·7	7·7	5·5	5·1	6·4	7·7	5·1	8·2	5·0	7·5	4·8	6·9	4·6	5·8	4·6	7·1	
24002	5·7	5·6	—	5·6	3·7	—	6·1	4·0	—	6·1	5·7	5·5	6·6	5·6	5·7	5·4	7·9	6·6	7·6	4·4	7·6	5·0	6·3	4·7	7·1	
24003	5·5	6·9	5·1	6·2	4·4	—	7·2	4·4	—	6·7	6·0	5·8	6·3	6·1	6·2	6·2	8·1	5·2	8·1	5·9	8·2	6·4	4·9	6·4	7·8	
Averages	5·4	6·2	5·3	5·6	4·6	—	6·4	4·3	—	6·0	6·7	6·0	6·0	6·3	7·0	6·5	8·3	6·3	7·7	5·6	7·9	5·5	6·2	5·1	7·3	

From the milligram equivalents it is obvious that the daily ration of food is potentially basic for all the groups to the extent of 399, 391, 363, 229, 123 c.c. normal solution for Batch A, B, C, Di, and Dii respectively. Milligram equivalents and " c.c. normal solution " are, of course, synonomous as explained by Theiler, Green, and Du Toit (1927) in " Minimum Mineral Requirements of Cattle." The acidity due to the CO_3 radicle of the ration is not taken into account, for, as stated by Marek (1929), carbonic acid is easily decomposed by another acid into carbon dioxide and water, and can therefore hardly be said to affect the acidity of the ration. The ratio of phosphorus to calcium varies for the different groups, but again it must be said that the authors do not attach great value to the dominant importance of fixed ratios of minerals in metabolism. Recent work by Becka (1929) supports this view. This author believes that magnesium is the regulator of calcium metabolism, but that neither the ratio of magnesium to calcium nor the total quantities present in the diet is important. The nature of the magnesium salts present, however, is of primary importance. Thus magnesium hydroxide " binds " calcium in the tissues, while magnesium sulphate favours its elimination. Becka applies this view, which is backed by experimental evidence to a study of osteomalacia with very fair success. In the absence of more experimental data on the importance of the ratios of minerals to one another the authors prefer to be open-minded and continue to regard the absolute intake of minerals of primary importance, while crediting the animal with more physiological capacity to adjust itself to varying ratios than some authors do.

During the pre-period, i.e. from 8/8/29 to 7/10/29, the animals were kept on the ration of Batch B.

Table II gives the weights of the sheep during the period under consideration.

The food consumption is shown in Table III.

The results of the blood analyses for the dates indicated are given in Table IV.

The inorganic phosphorus was determined as described in Green's paper, 1928. A slight modification of Roe and Rahn's method (1929) for the determination of calcium gave satisfactory results. The calcium determinations could unfortunately not be done from the beginning of the period due to pressure of other work. Then, too, it is not at all certain that calcium deficiency in the ration is reflected in the calcium level of the blood. Hence only a number of sheep have been bled for calcium determination, more of which will be included in the analyses as soon as positive results have been obtained.

DISCUSSION OF RESULTS.

Table II can be dismissed with a few remarks only. The outstanding feature of this table is the definite drop in the weights of the sheep in Batch A, whereas all the other batches gained in weight. It is remarkable too that the sheep in Batch B i.e. those on a low phosphorus ration but not extremely so, show good increase in weight. In this connection it must be remembered that one year old ewes are used in the experiment and are therefore, practically full-grown. Batch B may not show any deficiency of phosphorus

until lactation begins when the extra drain upon the bodies is bound to count. Besides, the animals are on a ration which is only slightly above maintenance hence phosphorus deficiency, unless extreme, can hardly be expected to affect the slow rate of increase in the weights of the sheep adversely in so short a period. The same argument would apply to the ewes in Batch Dii on a low calcium ration and sufficient phosphorus. Batch Di shows a poor increase in weight; even in individual cases this compares unfavourably with that of the sheep in the other groups, except A. The group is on normal calcium and excess phosphorus. Whether this fact has anything to do with the slight increase in weight of the group must be left for a later period when more data will be available and the experiment will have run for a longer time. The individual increases during the experimental period, as far as it has gone, are given for future reference when individual records can easily be studied.

Table III is interesting. First of all it confirms the observations of Theiler, Green and du Toit (1924) with regard to cattle that phosphorus deficiency decreases food consumption. Batch A shows this distinctly as there is an appreciable drop in the food consumption for the period December—January, viz. 20.5 therms as compared wth that for any of the previous periods. As in the other tables, individual records are given to enable the workers to study individual sheep over the entire period under experiment eventually. Further it must be noticed that Batch B is not characteristically different from C or D, although its sheep are on a ration low in phosphorus. It is still too early to risk conclusions, as the animals are probably getting sufficient phosphorus in their rations to meet the demands of their bodies which cannot be great for full-grown animals on practically a maintenance ration. Batches B, C and D require little comment. If the slight variations in the total food consumption per month have any significant bearing on the problem, this will be brought out more definitely later in the experiment.

A summary of the blood analyses is given in *Table IV*. This is perhaps the most interesting, for, in the light of "Mineral Metabolism and Blood Analysis by Malan" 1930, these figures lend themselves rather well to interpretations. The sheep in Batch A are on a ration extremely low in phosphorus and the average inorganic blood phosphorus of the group shows a very low figure viz. 2.3 mg. per 100 c.c. blood as compared with a normal figure of about 6.5. What is, however, more remarkable—and this applies to all the groups—is that 7 days after the beginning of the experimental period the sheep that were on a phosphorus deficient diet already showed a drop in the inorganic phosphorus of the blood. Batch A brings this out perhaps more clearly than any other group on account of its extreme phosphorus deficiency. The inorganic blood phosphorus is approximately 5 mg. for the preliminary period, but about a week after the beginning of the experimental period this figure already shows a decrease which lasts throughout the experimental period and becomes more significant from time to time. Batch B shows the same characteristic drop, but to a less marked degree. This work again brings out the observation made so often in cattle that in all but a negligible minority of cases—probably diseased—the inorganic phosphorus level of the blood may be taken as being

indicative of the degree of phosphorus deficiency of the animal. In this particular instance, although neither the food consumption nor the increase in weight suggests phosphorus deficiency in Batch B, a glance at the table giving the blood analysis shows clearly the symptoms of incipient aphosphorosis. This will probably be borne out by observations later on in the experiment.

Little need be said about the calcium content of the blood. As yet no characteristic differences are noticeable between the blood of Batch Dii on a diet low in calcium and that of the other groups. Results are eagerly awaited as to whether blood analysis is any aid in the study of calcium deficiency. This must be left for later work.

SUMMARY.

1. A detailed description is given of an experiment in progress for the determination of the rôle of phosphorus in the nutrition of sheep and the production of wool.

2. The sheep in Batch A, i.e. ration extremely low in phosphorus have decreased in weight, whereas all the other groups show an increase.

3. Extreme phosphorus deficiency affects food consumptions adversely.

4. The phosphorus intake of sheep is reflected in the inorganic phosphorus fraction of the blood and hence the value of blood analysis for phosphorus in the study of aphosphorosis is emphasized.

5. Figures are given for the calcium content of the blood of sheep.

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Studies in Mineral Metabolism.—XIII.

THE PHOSPHORUS PARTITION OF THE BLOOD OF SOME ANIMALS.

By A. I. MALAN, M.Sc., Biochemist, Onderstepoort.

In the article "The Unknown Phosphorus Fraction of Calf Blood," by Malan and Green (1928) the complete phosphorus partition of fowl blood is given and the unknown or nuclein fraction in both calf and fowl blood is definitely shown to be associated with nucleated material. In the course of that investigation blood from a variety of animals was analyzed for comparison from time to time, and these results are given in the present article.

The analyses were made according to the method described in Green's paper (1928).

Table I shows the phosphorus partitions in mg. per 100 c.c. of the blood of a representative from each of the orders Amphibia, Reptilia, Avia and Mammalia:—

Table I.

Phosphorus in mg. per 100 c.c. whole blood.

	Frog (Rana Esculanta).	Snake (Puff-adder).	Domestic Fowl (Leghorn).	Bovine (Lactating Cow).
Total Phosphorus	128·0	64·2	94·5	17·8
Lipoid P.	24·0	10·2	12·6	9·6
Organic Acid Sol. P.	37·9	29·1	33·9	3·5
Inorganic P.	8·5	7·7	2·1	4·7
Nucleoprotein	57·6	17·2	45·9	0

One factor stands out clearly in the above results, viz. the presence of nucleoprotein in the blood of animals whose red blood corpuscles are nucleated. It follows from this that the total phosphorus in the blood of Amphibians, reptiles, birds (and fishes) is higher, as one would expect, than that of mammals whose red blood corpuscles are not nucleated. How the different phosphorus fractions vary in the blood of animals belonging to the same order or whether related individuals show any similarity in their blood phosphorus partitions is impossible to say without further work. In the case of mammals, however, some light is thrown upon this point by the results given in Table II, and to a less extent in the case of birds. The phosphorus partitions of frog and snake blood are included for the sake of comparison.

TABLE II.

Phosphorus given in mg. per 100 *c.c. blood.*

Animals....	Frog.	Turtle.	Snake.	Pigeon.	Pigeon.	Fowl.	Ostrich.	Man.
Total P.....	128·0	67·2	64·2	189·0	224·0	91·0	109·0	32·5
Lipoid P.....	24·0	12·5	10·2	24·8	30·0	11·2	11·3	10·5
Org. Acid Sol. P.	37·9	26·3	29·1	50·4	56·9	30·5	39·0	19·3
Inorganic P.....	8·5	8·7	7·7	1·6	1·3	2·0	5·5	2·5
Nucleoprotein...	67·6	19·7	17·2	112·2	135·8	47·3	53·2	—

Animals....	Baboon.	Horse.	Mule.	Donkey.	Bovine.	Merino Sheep.	Barbary Sheep.	Swiss Goat.
Total P.....	36·8	27·5	27·7	33·6	17·6	17·2	20·5	19·4
Lipoid P.....	10·3	9·7	10·4	16·9	9·5	9·4	10·8	9·4
Org. Acid Sol. P.	23·0	15·6	13·7	13·6	3·3	3·2	4·6	3·8
Inorganic P.....	3·1	1·9	3·1	2·6	4·8	4·6	5·1	4·8
Nucleoprotein...	0·4	0·3	0·5	0·5	—	—	—	1·4

Animals....	Thor Goat.	Llama.	Kangaroo.	Dog.	Cat.	Rat.	Rabbit.	Guinea-pig.
Total P.....	19·8	42·0	37·5	43·6	26·5	41·0	48·0	35·0
Lipoid P.....	8·4	9·6	10·0	14·6	14·9	9·9	13·8	8·1
Org. Acid Sol. P.	4·4	20·9	22·9	22·2	5·7	25·3	30·9	20·8
Inorganic P.....	7·0	10·7	4·3	3·2	4·1	4·0	3·5	3·8
Nucleoprotein...	—	0·8	0·3	3·6	1·8	1·8	0·2	2·3

Greenwald (1913, 1915, 1925) believes that nucleoprotein may be present normally in mammalian blood; some of the results in Table II certainly strengthen this view. Abderhalden (1898) found nuclein in horse blood. The author has never been able to establish the definite existence of such a fraction in normal horse blood, although it must be said—and this applies to bovine and sheep blood as well—that in the great majority of cases the total acid soluble phosphorus and the lipoid phosphorus do not total up to the figure obtained for total phosphorus but slightly less. The difference is usually in the order of .5 mg. and although experimental error (±1.5 per cent.) is a possibility it can almost be ruled out for hardly ever does one obtain a figure approximately .5 mg. greater than that obtained for total phosphorus which, obviously, would happen in many of the determinations if the difference were due to experimental error only. The nucleoprotein fraction registered as this difference is usually small, but its existence is unquestionable in some cases of normal mammalian blood.

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Micro-Method for the Determination of Antimony in Organic Material, and of its Course through the Animal Body.

By J. S. OTTO, M.Sc., Research Officer, Onderstepoort.

(Thesis approved as extra credit for M.Sc. Chemistry, in the University of South Africa).

Potassium Antimony Tartrate has been in use for some years in Zululand for the treatment of bovine trypanosomiasis. More recently considerable success has attended the use of Antimosan for the treatment of this disease. It was considered that information in connection with the absorption, deposition and elimination of this antimony compound would be of some value in determining the mode of action of this drug. For accurate determination of small quantities of antimony it was found necessary to devise a new method of analysis. It is chiefly the description of this method and the experiments run in connection with it that is described in this paper.

The estimation of small quantities of antimony in biological material was found to be no easy matter. From the available literature no method of analysis for the determination of small quantities of antimony in organic material seemed to be suitable for the purpose. Adaptations of the methods of Beam and Freak (1919), and Treadwell and Hall (1911) for determination of antimony were found impracticable, the method of Boyd and Roy (1929) was brought to the writer's notice after the completion of the analysis recorded in this paper.

METHOD.

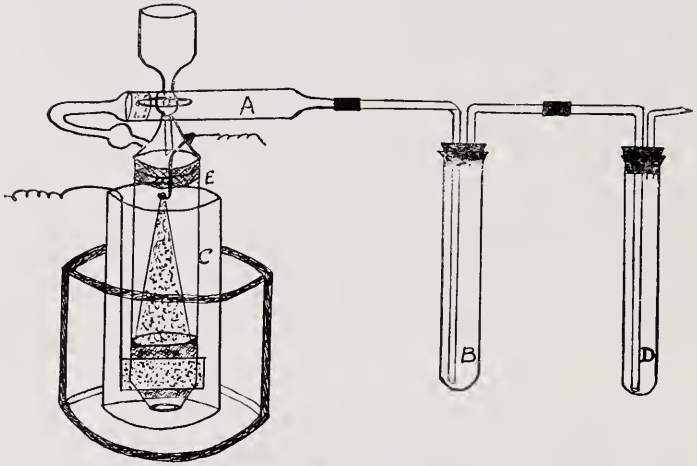
This method which is both reliable and expeditious, is based on the precipitation of Antimony as Ag_3Sb in a solution of silver nitrate of known concentration. The excess silver nitrate is estimated by titration against the Potassium sulphocyanide. This is a modification of Green's method (1918) for the determination of Arsenic in biological material. Antimony was determined in blood, liver, kidney and urine.

DESTRUCTION OF ORGANIC MATERIAL.

Fifty to seventy-five c.c. blood are measured into a porcelain basin of 100-150 c.c. capacity. For organs 50-75 grams are sufficient. Seven c.c. of a 20 per cent. Magnesium nitrate solution are added. The basin with its contents is placed on the steam bath for 2 hours, when it is transferred to a gas flame oven, where it is kept for 2 hours at a temperature rising from 100° to 250 °C. The contents of

the basin are then burned in an electric muffle to a white ash free from organic material, the temperature being raised to a dull red heat. The Magnesium nitrate prevents increase of bulk during the combustion process, while acting at the same time as an oxidising reagent. The most stable oxide of antimony, which is the one most likely to form during combustion, volatilses at 1560° C, vide V. and C. Meyer 1879. Hence there need be no fear of loss due to volatilisation during combustion.

APPARATUS.



For the production of nascent hydrogen electrolytically the apparatus of Moir and Jamieson (1925) is used with the slight modification that the inner glass tube and the cadmium electrode are replaced by a similar tube and electrode 3.5 cm. shorter, in order to reduce the total volume of the solution containing the antimony. A current of 3.5 amps is used at 6-8 volts, the current being kept constant by means of a rheostadt for 20 minutes. The time and current must be of a constant for all determinations. For each determination a fresh sample of cotton wool saturated with lead acetate solution, dried and kept in a dessicator, is used in the Tube A, for the absorption of volatile sulphides.

PROCEDURE.

Into the tube B 10 cm. x 1.5 cm., 6 c.c. of N/100 silver nitrate solution are measured accurately. The anode fluid in C must always be of the same concentration. 3.5 c.c. of sulphuric acid diluted sufficiently to fill the cathode tube to the mark E, are used as the solution in the cathode chamber. 3.5 c.c. of sulphuric acid are sufficient for dissolving the ash containing the antimony after combustion. After the addition of sulphuric acid, the ash is heated strongly to decompose any nitrate which may still be present and which interferes with the reaction. When cool the contents of the basin are diluted and washed into the cathode tube. Standard stock solutions N/10

$AgNO_3$ and N/20 KCNS are prepared, ferric alum being used as indicator. From these stock solutions, N/100 $AgNO_3$ and N/200 KCNS are prepared when required. 6 c.c. of N/100 $AgNO_3$ solution are placed in tube B. D also contains standard $AgNO_3$ solution to indicate whether antimony escapes B.

A blank is made on the reagents. The tube B containing the N/100 $AgNO_3$ solution is disconnected, and the contents filtered through a funnel, fitted with filter paper of good quality e.g. Schleicher and Schüll No. 595.

Five c.c. of the filtrate are then transferred to a 40 c.c. flask (using a standard pipette), and titrated from a small standard burette against N/200 KCNS, three drops of ferric alum indicator being used.

Solutions containing known quantities of antimony, ranging from .05 mg. to .3 mg. are then treated in exactly the same way as the blank. In each case the difference between the amount of N/200 KCNS required for the blank, and for a known amount of antimony is noted.

The quantity of antimony used is plotted against the number of c.c. N/200 KCNS which it requires.

It is surprising to find that the ratio of c.c. KCNS to mg. Sb is not constant, but very gradually increases when using from .05 mg. Sb to 5 mg. Sb. On the average .05 mg. is equivalent to .4 c.c. N/200 KCNS, as long as the amount of Sb remains relatively small. If the amount of Sb is more than would be liberated by the apparatus in the constant time, the current or the time must be increased. Further, if more Sb is present than would be precipitated quantitatively by 6 c.c. N/100 $AgNO_3$ a stronger solution of $AgNO_3$ must be used.

If due precautions are taken, consistent results are obtained, and, in determining amounts of antimony not less than .1 mg., the error is relatively small, although the percentage error is considerable at the lower limits.

It is, however, unnecessary to submit this particular curve, as the ratio aforementioned is not a constant, but depends on the rate of evolution of hydrogen which is a function of amperage, size of electrodes, and concentration of cathode and anode fluids. These vary, of course, for conditions differing from those mentioned above.

ABSORPTION OF ANTIMONY FROM THE BLOOD BY THE ANIMAL BODY.

EXPERIMENT No. 1.

Bovine No. 3511. *Black heifer.* *Weight* 250 *lb.*

Injected intravenously with 25 c.c. of 12 per cent. Antimosan containing 12.5 per cent. Antimony, i.e. 375 mg. Antimony.

Time.									
10 a.m.	Injected.								
10.7 a.m.	1st sample	blood	A	drawn	contained	·38	mg.	Sb.	per 100 c.c.
10.15 a.m.	2nd	,,	B	,,	,,	·26	,,	,,	
10.30 a.m.	3rd	,,	C	,,	,,	·23	,,	,,	
11 a.m.	4th	,,	D	,,	,,	·15	,,	,,	
12 noon	5th	,,	E	,,	,,	(?)	,,	,,	
20 c.c. urine gave ·3 mg. Sb.									

From this experiment we see that antimosan disappears from the blood very rapidly, about .9 of the Sb having left the blood within

seven minutes after injection. Thereafter the rate at which it leaves the circulation is much slower. It is possible that this reduction in the rate of removal from the blood is really due to the fact that after the first few minutes some of the removed drug is returned to the circulation. For example, it may be that the major part of the antimosan injected is picked up by the liver, and some of it then is passed back into the blood stream.

Urine was also collected from the same animal in a vessel, about 30 minutes after the injection of the antimosan, a normal amount being passed by the animal. As the bladder had not been drawn just before the injection no definite conclusions can be drawn as to the total amount of Sb present in the bladder. However, an analysis was made of the small quantity of urine collected; this gave a result of 1.5 mg. Sb per 100 c.c. which demonstrates that antimony is excreted via the kidneys, and appears in the urine within a short time after administration.

EXPERIMENT No. 2.

A further study of excretion was made by collecting and analysing the urine passed by a healthy human adult after intravenous injection of a small medicinal dose (H.E.H. 21/10/29).

Injected 5 c.c. of a 5 per cent. antimosan solution containing 31 mg. Sb intravenously into a man.

Time.	*Urine excreted.*	*Sb. per 100 c.c.*	*Sb. total.*
First 12 hours.....	840 c.c.	·075 mg.	·63 mg.
Second 12 hours.....	435 c.c.	·02 mg.	·088 mg.
Second 24 hours.....	1,500 c.c.	·009 mg.	·135 mg.
Third 24 hours.....	2,200 c.c.	·005 mg.	·110 mg.
Fourth 24 hours.....	1,450 c.c.	·0035 mg.	·051 mg.

During the first twelve hours 2 per cent. of the total antimony administered was eliminated whereas during the second twelve hours the percentage dropped to .3 per cent. This considerable drop in the rate of elimination was, however, not maintained for during the next three periods of twenty-four hours each the elimination was more or less on a par.

As it is shown in experiment 4 that a considerable portion of the antimony is retained by the liver, it would appear that there is an elimination, soon after administration, of part of the antimony which is in circulation. Thereafter, elimination at slow rate of some of the liver retained antimony continues. According to Georg Bock (1927) who worked on rats no antimosan was found in the urine twenty-four hours after injection.

EXPERIMENT No. 3.

Absorption after subcutaneous injection.

I. *Bovine* 3520; small red heifer; weight 250-300 lb.

II. *Bovine* 3502; small red and white ox; weight 250-300 lb.

Injected subcutaneously 25 c.c. of 12 per cent. antimosan into each animal.

Time.					
9.10 a.m.....	Injected.				
9.40 a.m.....	1st	sample	blood	A	drawn.
10.10 a.m.....	2nd	,,	,,	B	,,
10.40 a.m.....	3rd	,,	,,	C	,,
11.10 a.m.....	4th	,,	,,	D	,,
12.10 p.m.....	5th	,,	,,	E	,,
2.10 p.m.....	6th	,,	,,	F	,,

No antimony could be detected in any of these samples, i.e. at no period did the blood contain .05 mg. Sb per 100 c.c. blood.

III. Bovine 3511. Injected subcutaneously 25 c.c. of a 12 per cent. antimosan.

Time.								
9 a.m.....	Injected.							
9.6 a.m.....	1st sample	blood	A	drawn	contained	·03	mg. Sb. per 100 c.c. blood.	
9.15 a.m.....	2nd „	„	B	„	„	·055	„	„
9.30 a.m.....	3rd „	„	C	„	„	·025	„	„

IV. Experiment No. III repeated with same animal 14 days later.

Time.								
9 a.m.....	Injected.							
9.6 a.m.....	1st sample	blood	A	drawn	contained	·00	Sb. per 100 c.c. blood.	
9.15 a.m.....	2nd „	„	B	„	„	·018	„	„
9.30 a.m.....	3rd „	„	C	„	„	·00	„	„

The results of the analyses of the blood samples after subcutaneous injection were rather surprising. The rate of absorption appears to be so rapid that the highest concentration in the blood is reached 15 minutes after injection, but even this is very low. Nevertheless, it is probable that after subcutaneous injection, the greater portion of the drug does pass into the blood stream, but at a rate which allows its removal from the blood to be almost as rapid as its entry. That antimony is picked up by the liver after subcutaneous injection of antimosan, is shown by the following experiment.

EXPERIMENT No. 4.

Equine No. 18039.

10 a.m. An injection of 20 c.c. of a 12 per cent. antimosan given subcutaneously, slowly and with gentle massage at a point about 22 cm. below external angle of left ilium.

11 a.m. A similar injection given subcutaneously in front of left shoulder.

12 noon. A similar injection given subcutaneously in front of right shoulder.

1 p.m. A similar injection given subcutaneously in left flank.

2 p.m. The horse was destroyed. Incisions through the skin at the sites of the injections revealed neither appreciable inflammatory thickening nor any traces of unabsorbed drug. The two precrural lymphatic glands were dissected from their surrounding fat for chemical analysis.

Analysis of organs.

Organ.	*Weight.*	*Sb. per 100 gm.*	*Total Sb.*
Liver.....	3,350 gm.	·84 mg.	28·1 mg.
Kidney.....	760 gm.	·04 mg.	·31 mg.
Right precrural gland.....	2·24 gm.	—	·013 mg.
Left precrural gland.....	2·17 gm.	—	minute trace ·004 mg.

Another object of the experiment with horse 18039 was to ascertain if absorption was directly into blood vessels or into the lymphatics. The fact that there was only a minute trace in the left gland (in the neighbourhood of which an injection was made four

hours previously), and a somewhat larger amount in the right gland (injected one hour previously), indicates that some, at any rate, of the drug is absorbed via the lymphatics, but that it is not detained in the glands. To aid absorption by the subcutaneous route, a site for injection should be chosen which is well supplied by lymphatic vessels.

Also it is seen that a great amount of the drug is stored by the liver, while the kidneys contain a fair portion.

SUMMARY.

1. A method for the determination of Sb in biological material is described in detail.

2. From the experiments described it is concluded that Sb accumulates in the liver when antimosan is injected intravenously or subcutaneously.

3. Absorption of Sb takes place via the blood vessels and the lymphatics when antimosan is injected subcutaneously.

4. Part of the Sb is excreted by the kidneys after injection of antimosan.

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Section VI.

Pathology.

P. J. J. Fourie and T. Ziehn — A Study of a Case of Aleucaemia in the Dog.

J. Quinlan ... ... — Cystic Osteo-Chondroma of the Testicle in a Stallion.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

A Study of a Case of Aleucaemia in the Dog.

By P. J. J. FOURIE, M.R.C.V.S., Veterinary Research Officer, Onderstepoort, and T. ZIEHN, Veterinary Practitioner, Pretoria.

According to Hutyra and Marek (1926), pseudoleucaemia is generally regarded as a not infrequent disease of dogs and other mammals, but very few cases have been encountered in canines examined at Onderstepoort.* It is possible that one case in which a diagnosis of sarcoma was made from the histological examination of routine sections, was really a case of leucaemia, but unfortunately blood and organ smears were not available for haematological and microscopic examination. As far as we are aware a clinical and pathological description of the disease as it occurs in dogs in South Africa has not yet been recorded. In the case under discussion the second named author (Ziehn) was responsible for the clinical examination while the first named (Fourie) undertook the pathological study.

The subject of this paper was a 9½ year old pure-bred Irish setter bitch, which had won several championships. She had reared several litters of pups and there was no record of any serious illness during her life time. On the 24th January, 1930, the owner noticed a swelling under the throat of the bitch, and consulted one of us (Ziehn). On clinical examination all the superficial lymphatic glands of the body were observed to be symmetrically enlarged, but these were not painful. The spleen, on palpation, was found to be enlarged. The bitch had a peculiar stiff gait, the temperature was 106° F., the pulse was weak and frequent, and the respirations were somewhat laboured. The owner noticed an occasional cough. There was a small circumscribed apparently subcutaneous swelling in the region of the mammary gland. During the next few days the temperature fluctuated between 103° F. and 106° F., but the general weakness progressively increased. Although a microscopic examination of blood smears did not reveal any striking changes—no differential counts were made at the time—a tentative clinical diagnosis of lymphatic leucaemia was nevertheless made (Ziehn). It was felt, however, that malignant tumours, particularly sarcomata, could only be definitely excluded by positive haematological findings.

On the 27th of January the patient was brought to Onderstepoort for further examination. She was at this time very weak and could not stand. Blood was collected in the usual way from the jugular and recurrent tarsal veins, .1 c.c. of a 7.5 per cent. sodium citrate solution being used as anticoagulant for each c.c. of blood. The red and white counts were made in the usual way and the per-

* Since this article went into press the writers came across a further case in an aged cross bred Pekinese dog, showing typical clinical symptoms of a lymphatic aleucaemia. The Red count was 8 millions and the White count 5,000 per c.mm. of blood. Numerous immature cells of the lymphoblast type were present in blood smears. This case was unfortunately not available for Pathological examination.

centage haemoglobin was determined in a Duboscq colorimeter, a Newcomer disc being used. The percentage volume of red cells was determined by centrifugalisation at a rate of 1,500 revolutions per minute. The figures obtained were: Red count, 5.3 millions per c.mm., Haemoglobin, 87 per cent., percentage volume 25, total white count, 3,000 per c.mm.

In counting 700 cells the lymphocytes were present to the extent of 77 per cent. The lymphocytes were mostly adult cells, but a few of them were undoubtedly immature cells of the lymphoblast type having a pachychromatic nucleus with one or two nucleoli. (Figure 1.) The remaining 23 per cent. of leucocytes consisted of neutrophiles and monocytes. In some cases these were rather difficult to classify definitely as, many being young forms, the typical differential features had not yet developed. The probability is that most of them were neutrophiles. There were quite a number of normoblasts present. The bitch died on the 30th January, and a post-mortem examination was made about two hours after death.

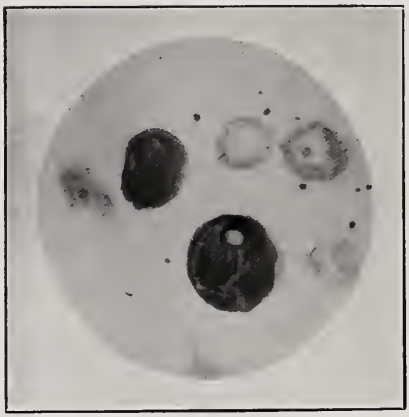


Fig. 1.
Cell of lymphoblast type.
1400 ×.

The condition of the animal was good, rigor-mortis was not complete, the blood stained moderately, and had not coagulated. On the ventral portion of the abdomen, situated partly subcutaneously and partly in the mammary glands there was a circumscribed swelling measuring 1 cm. in diameter. Further macroscopic post-mortem observations together with the microscopic examinations are described under the headings of the various organs.

Mammary Tumour.

On section this cut rather hard, was of a grey colour being somewhat opaque towards the centre but translucent towards the periphery.

Microscopic Examination.—The centre consisted mainly of round cells, with large vesicular nuclei, which contained one or more darkly staining (with haemalum eosin) bodies. These were interpreted as nucleoli. From the appearance of these cells in sections, it was found difficult to classify them definitely, but they resembled cells, probably lymphoblasts, seen in large numbers in the bone-marrow of this animal. A smaller number of neutrophiles and eosinophile myelocytes were also present. In the immediate vicinity of this central group of cells [Figure 2(1)] there was a small area

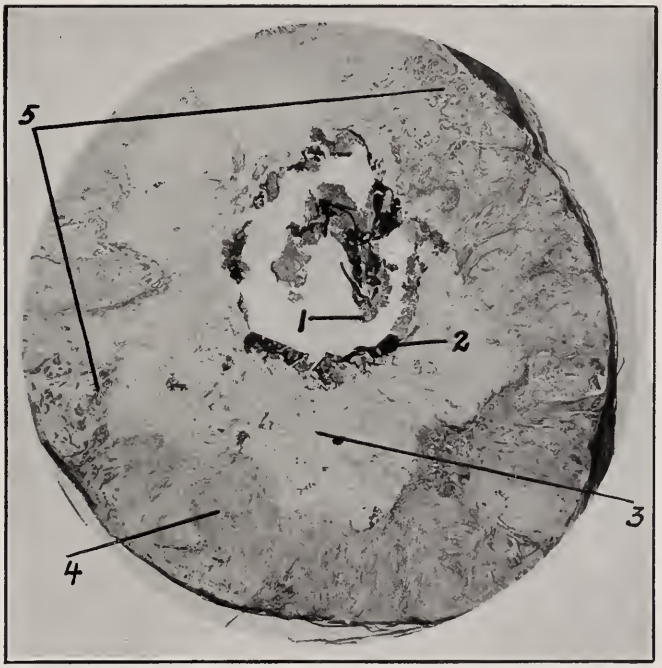


Fig. 2.

Osteo-chondro-sarcoma in Mammary gland. (12 ×.)
(1) Marrow; (2) Bone; (3) Cartilage; (4) Bundle of spindle cells; (5) Mammary epithelium.

where apparently partial formation of bone (with cartilage cells) had taken place [Figure 2(2)]. This was surrounded by a fairly wide zone in which cartilage cells were frequent [Figure 2(3)]. The periphery of the growth consisted of bundles of spindle-shaped cells, with a fair amount of fibrous tissue between the bundles [Figure 2(4)]. In this peripheral portion one could recognize here and there the remains of mammary epithelium [Figure 2(5)]. The tumour was identified as an osteo-chondro-sarcoma, which had developed bone-marrow. It is remarkable that this bonemarrow also showed pathological changes similar to those to be described in the marrow of the normal bones of this animal.

Lymphatic Glands.

These were bilaterally enlarged. The popliteal glands measured 4 by 2.5 cm., the mandibular glands 5 by 4 cm., the dorsal prescapular 5 by 3.5 cm. and the ventral prescapular 4.5 by 3 cm. On section all the glands presented more or less the same macroscopic appearance, there being discrete areas of a red colour in a background of grey. The red areas which were irregularly scattered throughout the substance of the glands had an approximate cross measurement of 2 mm., and were soft in consistence. The bronchial glands were enlarged and markedly pigmented, the mediastinal glands were slightly pigmented. The peripartal glands were

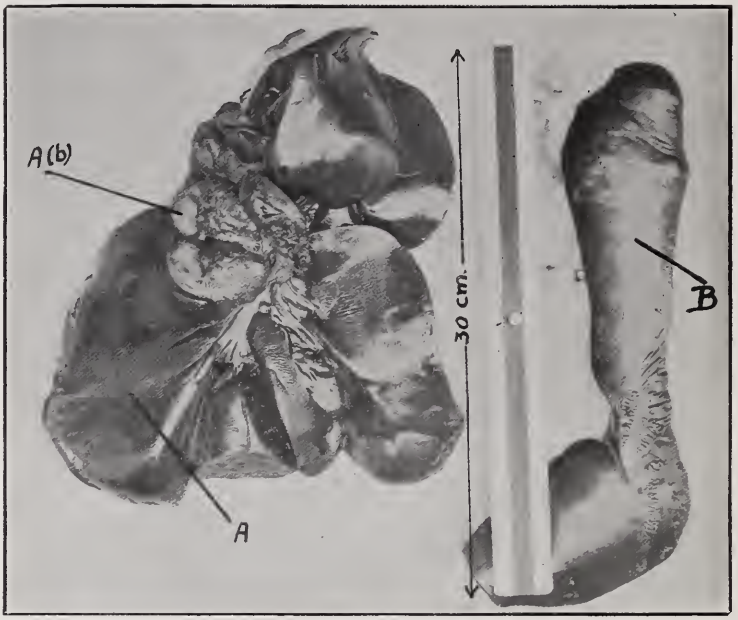


Fig. 3.

A Liver; A (*b*) Fibrinous gelatinous mass in portal lymphatic gland; B Spleen.

very much enlarged (Figure 3), the largest node measuring 6.5 by 4 cm. In the ventral half of this node there was a circumscribed and well defined gelatinous mass measuring 1.7 by 2.5 cm. [Figure 3A(*b*)]. A similar, but smaller area was present towards the centre of the gland. The mesenteric glands were also enlarged, and on section were moist and varied in colour from grey to slatey blue.

Microscopic Examination.—The mesenteric, peripartal prescapular, popliteal, bronchial and mediastinal glands were examined. In some glands no marked changes were present, but in others,

especially the periportal, prescapular, popliteal and mesenteric glands, the normal structure was completely lost. There was marked hyperaemia and in places extensive haemorrhage. In some glands, especially the periportals and prescapulars, there were areas up to 1 mm. in diameter, which consisted of a network of fibrinous filaments (Figures 4 and 5), in some cases unassociated with any cellular elements, but in other cases with the presence of red cells. This gave one the impression that the lesion was an acute fibrinous lymphadenitis. No neutrophiles were present in any of these lesions, but round cells were sometimes associated with red cells and fibrin. Some of these round cells had undergone, or were undergoing, necrosis. The probability is that the fibrin was not of an inflammatory nature, but had developed as a result of haemorrhage. In view, however, of the absolute dearth of neutrophiles, to be described later in connection with the bonemarrow, one would hardly expect to find an inflammatory exudate of neutrophiles. The areas where these collections of fibrin were present were probably structurally the same as those much larger areas described macroscopically in the periportal glands. There was in addition well marked lymphoid hyperplasia in all the glands and in some there were well defined perivascular lesions (Figure 7), which when cut longitudinally, extended in the form of

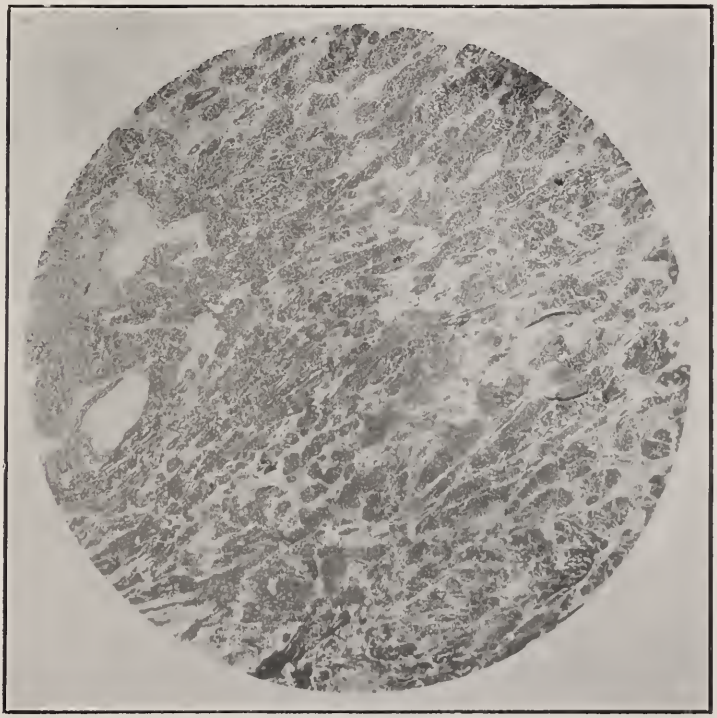
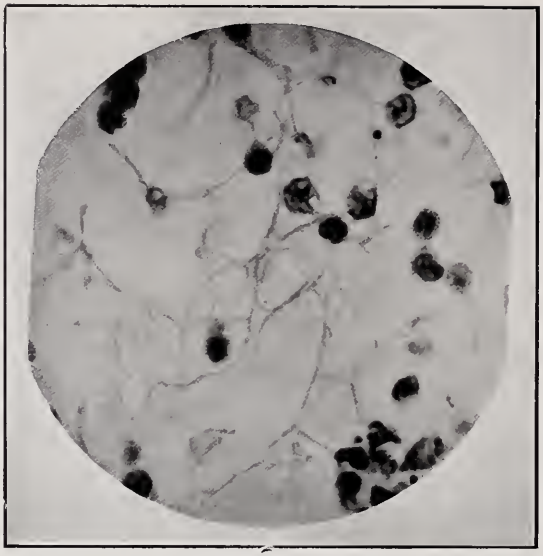


FIG. 4.
Periportal lymphatic gland. 12 ×.



As fig. 4,—showing fibrinous filaments. 1000 ×.

Fig. 6.
Prescapular lymphatic gland. 12 ×.

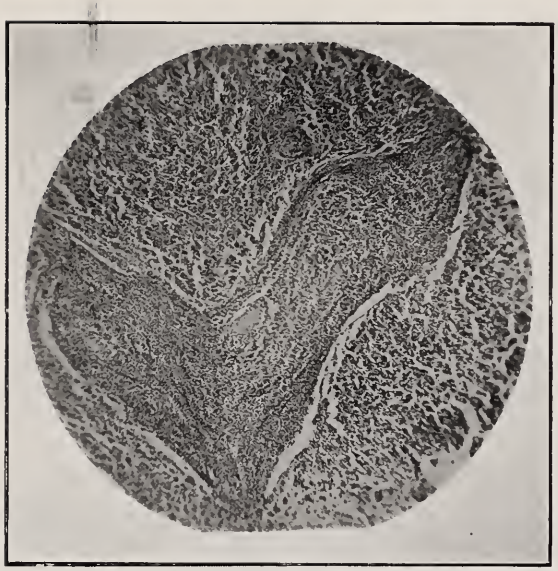


FIG. 7.
As fig. 6,—showing perivascular lymphoid hyperplasia. 50 ×.

thick strands from the cortex deep into the substance of the gland. In those that were cut transversely, the cells were seen in masses around one or more blood vessels. Many of the cells towards the periphery of these perivascular accumulations were in varying stages of necrobiosis. Except that these cells were perhaps more closely packed and that they were definitely situated perivascularly, there was no recognisable morphological difference in sections between them and the other lymphoid cells occurring in the gland. The extent of these perivascular lesions varied greatly in the different glands.

SPLEEN (Figures 8-12).

This organ was very much increased in size (Figure 3*b*). It measured 34 by 9 by 2 cm., and weighed 320 gm. On section a number of circumscribed foci, measuring 2 mm. in diameter, were seen to be irregularly distributed throughout the substance of the gland. These were probably enlarged malpighian bodies, and except for these, the malpighian bodies were otherwise not well defined. The trabeculae were indistinct. The pulp was of a reddish colour, somewhat soft in consistence and could, to some extent, be scraped from the cut surface.

Microscopic Examination.—Nodules of varying size occurred in an irregular manner throughout the sections examined. The nodules were all perivascularly situated. In some nodules the cells were closely packed in large numbers around the vessels. It was difficult to determine the exact structure of these fairly large round cells in

sections, but as far as could be made out, they occurred here unassociated with other cells. Towards the periphery of the nodule a large amount of blood was present. In some nodules the periphery was well defined, but in others red cells and round cells were freely intermingled at the periphery. There were even nodules in which it seemed that the blood was encroaching on them to such an extent that they were gradually being replaced by blood. Between the nodules the pulp consisted in places of large numbers of red cells, with relatively few other cells, but in other places round and other cells occurred diffusely in the pulp together with large numbers of red cells. Large giant cells, which were interpreted as megacaryocytes occurred almost in colonies at the periphery of some nodules [Figures 9, 10(*a*) and 12], especially where many red cells were at the same time present. These megacaryocytes were also seen irregularly scattered throughout the pulp. Where nodules were superficially situated, just under and in contact with the capsule, this was infiltrated with round cells. The trabeculae were not distinct. From the study of embedded sections, it was not possible to classify definitely all the cells seen in the pulp. Here and there in the pulp were

Fig. 8.
Spleen. 12 ×.

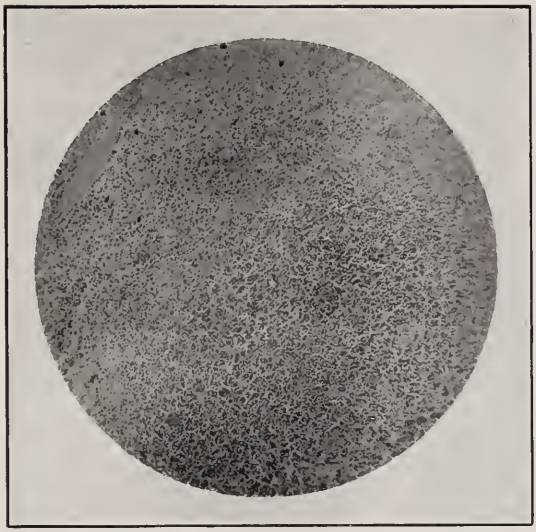


FIG. 9.
Periphery of a lymphoid nodule, dark bodies in pulp are megacaryocytes. 60 ×.

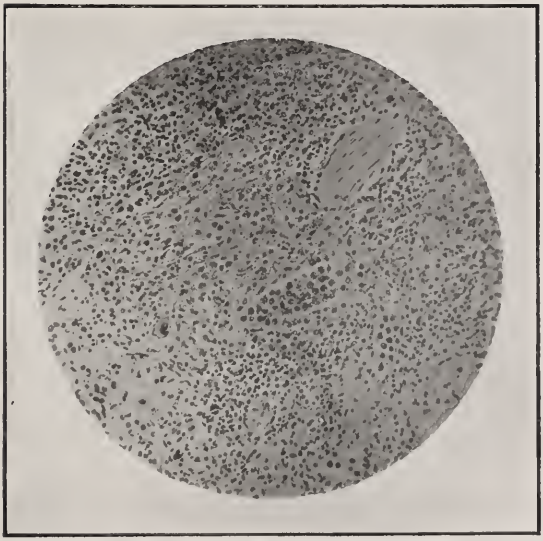


FIG. 10 (*a*).
Pulp with several blood islands—myeloid metaplasia. 120 ×.

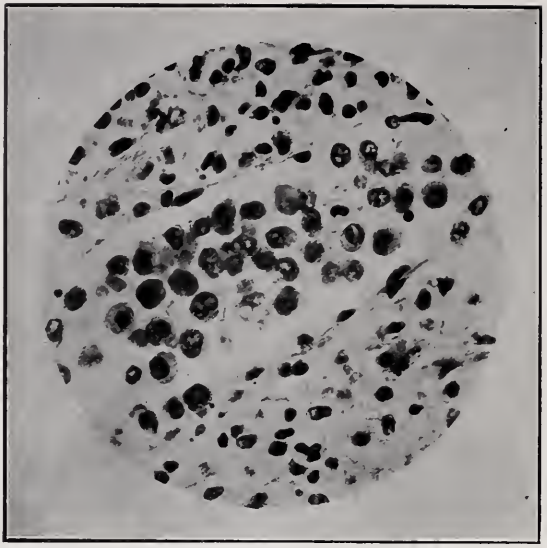


FIG. 10 (*b*).
As fig. 10(*a*) showing blood island with endothelium, and in centre megaloblasts, normoblasts and possibly also erythroblasts. 480 ×.

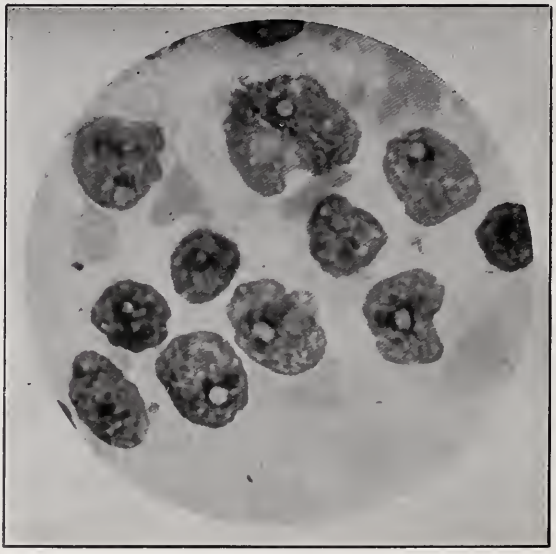


FIG. 11.
Immature cells from spleen smears. 1400 ×.

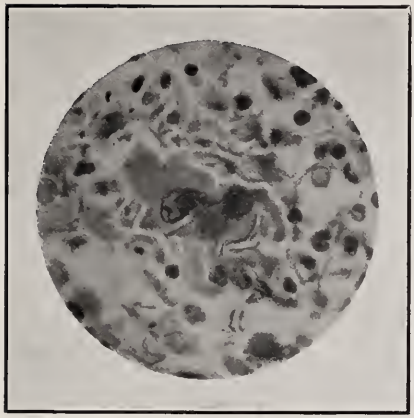


FIG. 12.
Megacaryocyte from embedded spleen sections. 600 ×.

peculiar groups of large cells. Some groups consisted only of a few cells, but in others quite a number of cells were present. These occupied spaces (primitive blood spaces) which were lined by flattened cells resembling endothelial cells. The nuclei of the cells varied in structure, some being vesicular, and others being darkly staining and pycnotic. These clusters of cells were interpreted as being analogous to the blood islands, which occur in early embryological development and later on in various organs, such as the liver and the spleen. If this is the correct interpretation, the cells would be primitive blood cells, the primitive lymphocytes or the lymphoblasts of Maximow, quoted by Bailey and Miller (1921), or the haemocytoblasts of Maximow (1928). Some of the cells were probably megaloblasts, and evidence that differentiation was occurring towards the erythrocyte. was found, as some cells had already developed nuclei, similar to those of normoblasts [Figure 10(*b*)] and even of erythroblasts. The nuclei of some of the cells in these so-called blood islands were in a state of mitotic division. There seems to be a good deal of confusion concerning the use of the term erythroblast. According to Arey (1924) it is often regarded as the final stage in the ontogenesis of the immature erythrocyte, just before the adult red cell is formed, whereas megaloblasts are sometimes also referred to as erythroblasts. It is not possible to make a definite statement, but one had the impression that the cytoplasm of these cells stained somewhat like that of red cells.

LUNGS.

The left lung was more or less collapsed, there were numerous dark grey areas on the surface. The right lung was partially collapsed. In the dorsal portion of the apical lobe of this lung was a large solid area which was raised above the surrounding surface. A good deal of fluid and froth could be scraped from the cut surface. The cardiac lobe of the left lung felt solid and liver-like, and from

it a good deal of fluid escaped on section. Numerous small circular grey foci, about 1 mm. in diameter were present in both lungs.

Microscopic Examination.—A good deal of pigment was present throughout the lungs. The pulmonary capillaries were distended with blood. The alveoli contained: (1) structureless serous fluid; (2) occasional catarrhal cells; (3) fibrin—in some alveoli only a few filaments were recognized, but in others the fibrin was more abundant and was seen more or less uniformly distributed in the form of a fine network; (4) numerous bacteria, mostly cocci, but bacillary forms could also be identified. In places circumscribed foci were seen, but it was not possible to determine definitely the nature of the cells in them. The nuclei were pycnotic and in some cases even disintegrated. These were apparently areas of commencing necrosis but it was not clear if these were the greyish foci seen macroscopically. No well defined perivascular or other accumulations of round cells were present. These lesions could be interpreted as early stages of an acute fibrinous pneumonia due to bacteria, with commencing necrosis in places and showing in addition anthracosis.

Liver (Figures 3 and 13-16).

This organ was markedly enlarged and weighed 1.8 Kgm. The borders were rounded, lobulation was very distinct. On section the liver cut moderately hard and a good deal of blood escaped from the cut surface. The intra-lobular tissues were of a greyish appearance and stood out prominently. The central veins seemed to be encroached upon by greyish foci, which gave the liver a mottled appearance.

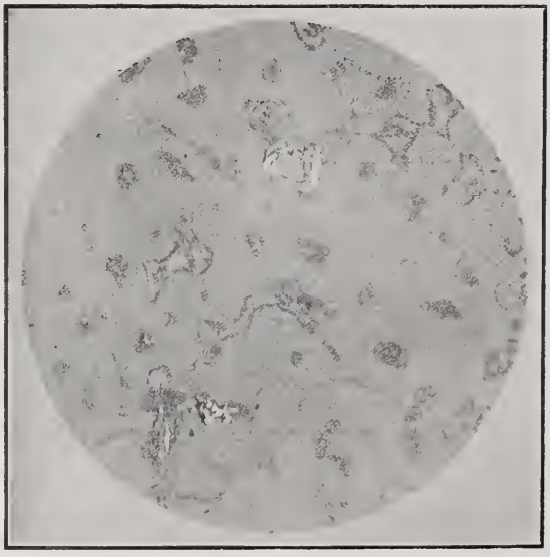


Fig. 13.
12 ×.

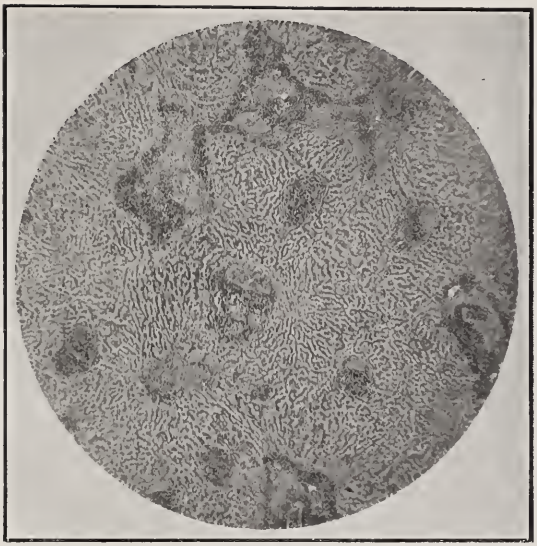


FIG. 14.
25 ×.

FIG. 15.
50 ×.

Figs. 13, 14 and 15. Sections of liver showing perivascular lymphoid hyperplasia and stasis atrophy.

FIG. 16.
Immature cells from liver smears. 1400 ×.

Microscopic Examination.—Well defined and characteristic lesions were recognized. There was a very marked perivascular accumulation of round cells of the lymphoid type, especially in Glisson's capsule. The type of cell seen was remarkably uniform throughout. A perivascular lymphoid hyperplasia seemed also to be associated with the central veins. The morphology of these cells was like that of the dominant cell to be described in the bonemarrow. These perivascular accumulations resembled those which occur in the liver in East Coast fever described by Steck (1928). Irregularly scattered throughout the liver were blood pools or lagoons and in some cases haemorrhages were actually present. There was a severe stasis, as a result of which the cell rows were pushed widely apart. The liver cells themselves showed degenerative changes. These lesions of the liver seemed to be secondary to the perivascular lymphoid hyperplasia, which encroached on the blood vessels and caused the stasis, with consequent atrophy of the liver cells and resulting eventually in actual haemorrhage. In addition to the stasis atrophy, the lymphoid hyperplasia caused destruction of the liver tissue by pressure (pressure atrophy). The liver was very carefully examined for myeloid tissue, but no evidence of this could be found.

BONEMARROW.

No well defined macroscopic changes were recognized, but very striking changes were found to be present on histological examination (Figures 17 and 18). Bonemarrow from the decalcified femur and ribs was embedded and the fatty bonemarrow (Figures 19-21) was

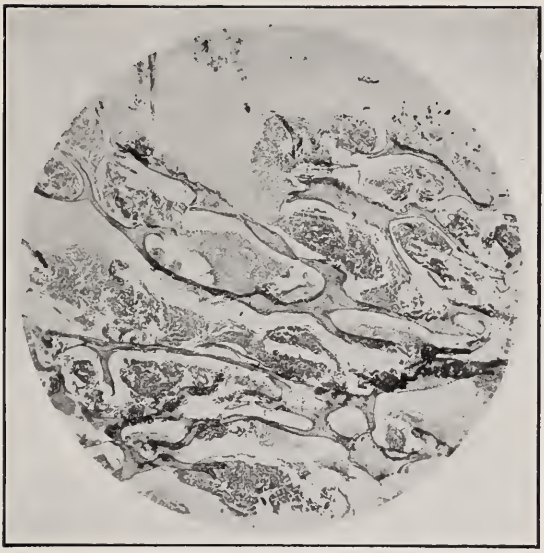


FIG. 17.
Bonemarrow. 12 ×.

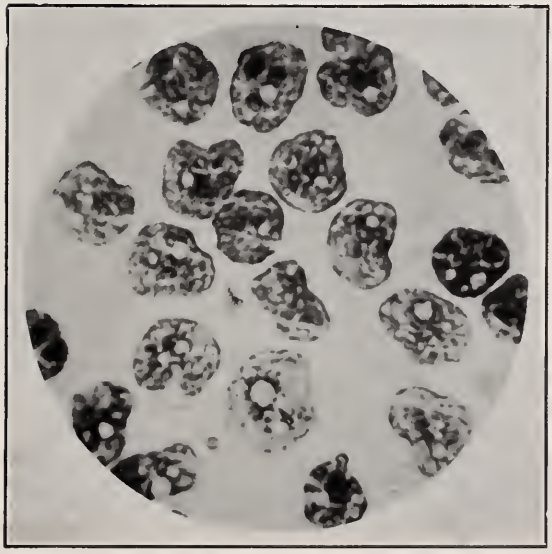


FIG. 18.
Immature cells of lymphoid type in varying stages of development from bonemarrow smears. 1400 ×.

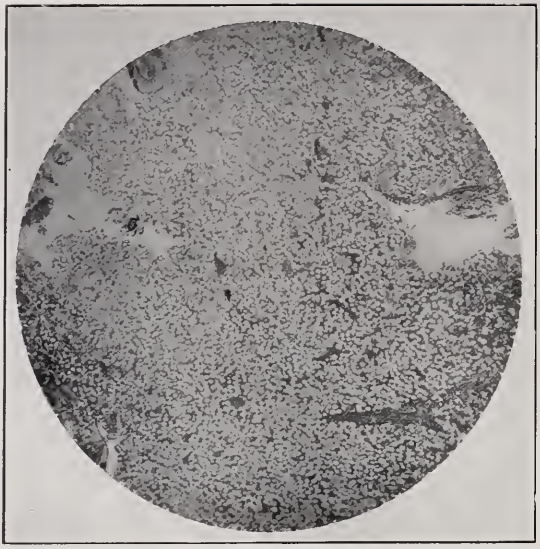


FIG. 19.
12 ×.

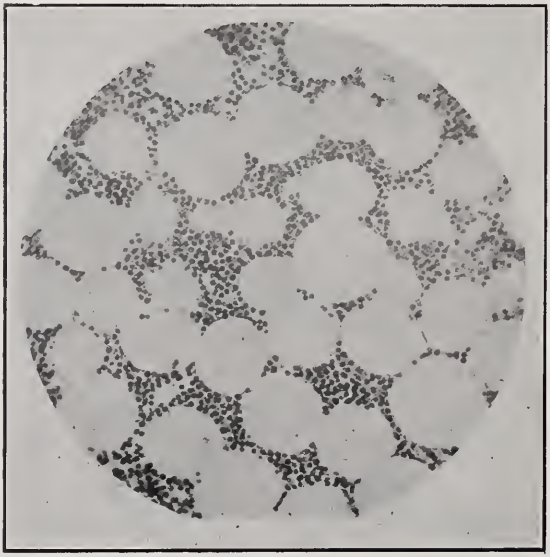


FIG. 20.
200 ×.

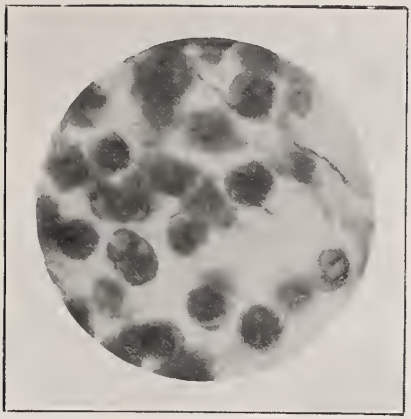


FIG. 21.
1400 ×.

FIG. 22.
600 ×.

Figs. 19, 20, 21 and 22. Embedded sections of fatty bonemarrow.

carefully scraped out with a curette, preserved in formalin and embedded. The granulocytes with their immediate precursors the myelocytes were relatively few in number. They were difficult to find and in many fields were altogether absent. The dominating cell, was a round cell. The exact structure of this cell was difficult to determine in sections, but it will be discussed in greater detail when

describing the organ smears. It was, however, a very immature type of cell, probably a myeloblast or a lymphoblast. Giant cells such as were seen in sections of the spleen were also present. There was hyperaemia of the fatty bonemarrow and very few granulocytes could be recognized. Even here there was evidence of perivascular infiltration of round cells.

Bladder.

Some straw coloured urine was present and the follicles were prominent macroscopically. The walls of the organ were very much thickened. In places there were well developed nodules of lymphoid tissue just under the mucous membrane. In parts of the organ these nodules had developed to such an extent, that the overlying mucous membrane had been completely destroyed, and the nodule, so to speak, actually protruded into the lumen of the organ. Where the round cells had not accumulated to the extent of actual formation of nodules, they were more or less diffusely present under the mucous membrane. The nodules themselves contained one or more blood vessels, around which mostly round cells were present. No giant cells were present in these lesions. Around many of the nodules were collections of red cells, which actually formed haemorrhages.

Gastro-intestinal Tract.

The contents of the stomach were liquid. Its mucous membrane was reddened in places. An ulcer was present towards the pylorus. The mucous membrane of the duodenum was swollen and reddened in places. A few tapeworms were present (Dipylidium sexcoronatum). There was thickening of the mucous membrane of the rectum, with well marked reddish discolouration towards the anus. In the walls of the rectum were small firm nodules. The lymphoid nature of these nodules was determined on microspic examination.

Kidneys.

There was slight hyperaemia and the glomeruli were fairly prominent, but no other lesions were found to be present on microscopic examination.

Heart.

The pericardial sac was empty. A few dark red spots were present on the epicardium. The right ventricle was in diastole and contained a large well formed blood clot. The endocardium, atrio-ventricular and pulmonary semilunar valves were normal. The left ventricle was in systole and contained a small blood-clot. The endocardium, atrio-ventricular and aortic semilunar valves were normal. The colour of the myocardium on section was a fairly uniform greyish brown. Its consistence was firm.

Microscopic Examination.—The myocard was normal, except in a few places, where there were slight accumulations of round cells. In the immediate vicinity of these small foci, round cells were found irregularly distributed amongst the muscle fibres. The interpretation of these cells was not quite clear. There might have been a slight subacute myocarditis but there was also the possibility that this represented the commencement of lesions of a leucaemic nature in the heart.

Adrenals.

Except for hyperaemia and slight haemorrhages in the cortex, no changes could be recognized.

The other organs were all apparently normal, including the nervous system which was specially examined.

A careful microscopic examination was made of smears from the blood, the spleen, the marrow and the liver. Blood from this dog was injected into other dogs, but these transmission experiments were negative.

Discussion.

In the blood smears very few granulocytes are present. No eosinophiles and basophiles are present. The nuclei of the neutrophiles contain only a few lobes, but according to Bunting (1928) there would not appear to be reliable evidence that the greater number of lobes or parts of the neutrophile nucleus is always a definite indication of age of this cell. Most of the lymphocytes are mature cells, but occasional young forms, and even some cells which can be regarded as lymphoblasts, were recognized (Figure 1). Taking 10,000 as the average normal number of leucocytes for the dog [Klieneberger (1927)], there is a definite leucopenia in this case with its total white count of only 3,000 leucocytes. It is interesting to note here, that in East Coast fever, which is described by Steck (1928) as lymphadenosis aleucaemica parasitaria, there is also a well marked leucopenia. According to Kitt (1927) variations in the blood picture may occur during the course of leucaemia. There may be no increase in the number of leucocytes in the earlier and later stages and finally towards the end of the disease the exhaustion of the bloodforming organs may even cause leucopenia. Kitt draws attention to the fact that although these are in reality different stages of the same disease they have been designated as sub-lymphatic leucaemia and lymphatic or myeloid aleucaemia. From the differential counts obtained (77 per cent. lymphocytes and 23 per cent. other cells), it is clear that the proportion of lymphocytes is enormously increased, at the expense of the neutrophiles. On calculating the total number of lymphocytes per c.mm. of blood, the figure 2,300 is obtained. The average total number of lymphocytes in the blood of normal dogs, calculated from figures supplied by Klieneberger (1927), is 1,500-1,600. It is debatable, if one is justified in regarding this as a slight absolute increase in the number of lymphocytes, or whether it falls within the limits of normal variation. If the latter is the case, the condition can be designated as a relative lymphocytosis, although MacCallum (1927) maintains that the term "lymphocytosis" may be properly used, even though the total number of leucocytes is not increased. The decrease in the number of neutrophiles is undoubtedly due to the pathological changes in the bonemarrow, and it is extremely unlikely that this could have been merely a temporary or transitory leucopenia. Unfortunately only one haematological examination could be made, as the bitch died before this could be repeated.

It is exceedingly difficult to find any granulocytes or myelocytes in smears made from the bonemarrow. There are a few cells having the structure of plasma cells. The characteristic cell which is present to the almost complete exclusion of the other usual marrow cells, is one, which is fairly large, has usually a pachychromatic nucleus, with one to two nucleoli, which may be quite large. Very little can be said of the cytoplasm of this cell, as it occurs in marrow smears,

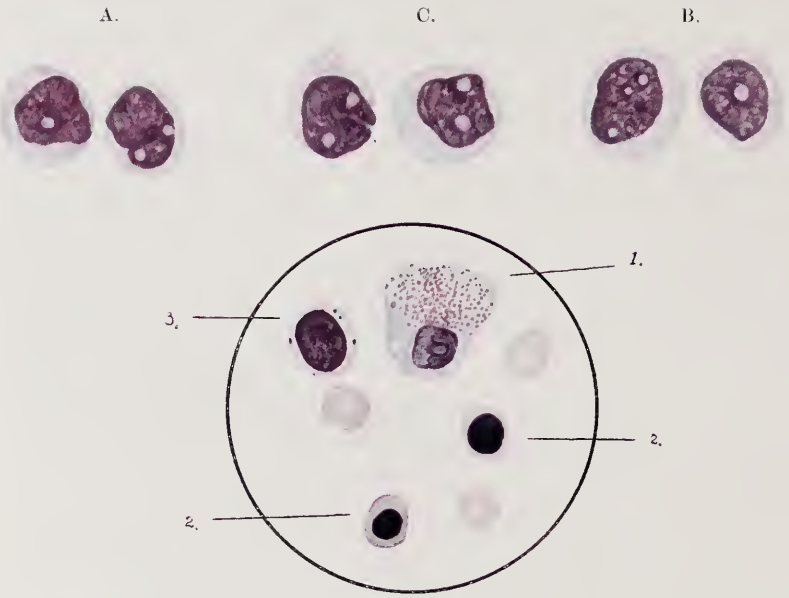


FIG. 23.

A.—Cells from liver smears.
B.—Cells from spleen smears.
C.—Cells from bonemarrow smears.
1.—Eosinophile myelocyte from spleen smear.
2.—Normoblasts from blood smear.
3.—Lymphocyte from blood smear.

as it did not stain very well, even with Pappenheim-May-Grünewald-Giemsa's method of staining. Some of the cells undoubtedly have leptochromatic nuclei and occasionally as many as 5 nucleoli are present. This cell has the structure of the haemocytoblast of the unitarians and seems to correspond more to the lymphoblast of the dualists, than to their myeloblast It is, however, not possible to classify it from the point of view of the dualists with any great degree of certainty. Unfortunately tests for the oxidase and other reactions were not made when the fresh material was available.

In liver smears the same type of cell is dominant. As a result of the tremendous stasis and pressure atrophy that the liver cells were subjected to, only very few intact liver cells were present. The same type of cell was found to be dominant in spleen smears, but on the whole they contained rather more nucleoli, than similar cells in the liver smears. This may have special significance and these cells may then really be myeloblasts, which is all the more likely, if the tissue of a myeloid nature described in the spleen, be considered, together with the presence of megacaryocytes and normoblasts in the spleen and also the presence of myelocytes to be referred to later. Downey (1928) quotes several cases where it was difficult or impossible to distinguish between genuine myeloblasts from the marrow and cells having a similar structure, but derived from lymphatic tissue and differentiating into lymphocytes. He quotes a number of authors who have had the same difficulty and concludes " that in acute leucaemia and in chronic myelogenous leucaemia, a cell form appears in the blood and tissues, which is termed a myeloblast, if the affection involves primarily the myeloid tissue, and a lymphoblast, if the disease is centred in the lymphatic tissue." Du Toit (1916) gave an exhaustive review and an interesting discussion of the monophyletic and polyphyletic theories. He states that it is not possible to make a rigid differentiation on morphological grounds between myeloblasts and lymphoblasts and that the cell described by Pappenheim as the lymphoidocyte, is the parent cell of lymphocytes and granulocytes.

With the extensive destruction of bonemarrow that had occurred in this case, one would have expected a severe degree of anaemia. This was not the case. There is, of course, a possibility that the blood may have been unduly concentrated at the time the animal was bled. This is merely mentioned as a theoretical possibility, but there is not the slightest evidence or suspicion that the animal refused water, which could account for such concentration of the blood. Diarrhoea was certainly not responsible, as this only occurred some days later, shortly before the animal died.

The lesions of the spleen are not typical of an advanced case of leucaemia, particularly if it be considered that the spleen was very much enlarged. Here and there definite macroscopic follicles are present, which on microscopic examination are clearly perivascular lymphoid accumulations, but these form only a relatively small proportion of the spleen. A large number of normoblasts were present in the blood smears, which is an indication of very active regeneration somewhere. The lesions of the bonemarrow are such that it is extremely unlikely that it could furnish such a large number of normoblasts. No other marked evidence of anaemia is present. In the spleen smears quite a number of normoblasts can be identified and basophile and eosinophile myelocytes [Figure 23

(1)] can be recognized, in addition to the megacaryocytes previously referred to. Cells like the eosinophile myelocyte reproduced were only rarely found, most of the cells being much younger forms, with granules just commencing to form. It can be stated with a fair degree of confidence that there are more myelocytes in the spleen than in the bonemarrow. Joest (1929) states that it is noteworthy that myelocytes are not seldom found in lesions of lymphatic leucaemia of the spleen, but the significance of these cells in leucaemia is not stated.

Naegeli (1923) mentions a large number of conditions in which myeloid metaplasia of the spleen takes place, and quotes various authors, who have shown that the condition occurs in lymphomatosis, in leucaemia, and where malignant tumours (carcinomata) involve the bonemarrow. When myeloid metaplasia takes place, the spleen is, according to Naegeli, one of the first organs in which this can be demonstrated. The presence of the normoblasts, the megacaryocytes, the myelocytes and particularly the blood islands [Figures 10(*a*) and 10(*b*)], is convincing evidence of myeloid tissue and indicates that the spleen is reverting to its embryological function in so far as the production of red cells is concerned, even if it is assumed that the haemocytoblasts described in the spleen cannot be definitely classed as myeloblasts. If this is the case the myeloid metaplasia is probably of a compensatory nature. This will explain the mild anaemia in spite of the extensive destruction of bonemarrow that had occurred. Assuming that this is the correct interpretation of the lesions in the spleen, the question which immediately arises is why this compensation was not also directed towards the production of granulocytes. The reply that suggests itself is, that the compensation is specialized and aims at maintaining the most urgent and vital requirements of the body. The severe destruction of the bonemarrow created the stimulus for the development of myeloid tissue and the spleen reverted to its embryological function in order to supply the necessary red cells. If the animal had lived long enough, the developing pneumonia in the lungs would possibly in its turn also have stimulated the spleen, to the production of an increased number of myelocytes and granulocytes; indeed, the very young myelocytes described, may be evidence that this was already taking place. If, in accordance with the monophyletic theory the lymphoidocyte of Pappenheim can differentiate into erythrocytes, lymphocytes and granulocytes, there seems to be no reason why myeloid tissue in the spleen cannot become specially adapted to the production of parent cells, which will differentiate mainly into erythrocytes at the demand of the economical needs of the body.

For comparative purposes the dominating cell in the bonemarrow, spleen and liver smears are reproduced side by side (Figure 23).

For convenience the complete anatomical pathological diagnosis may be summarized: Very marked symmetrical enlargement of the lymphatic glands throughout the body; hyperplasia of some of the lymphoid follicles of the spleen, with a development in the pulp of myeloid tissue of a compensatory nature; lymphoid hyperplasia of the bonemarrow, with consequent destruction of myelogenous tissue; very marked perivascular lymphoid hyperplasia in the liver, with stasis and pressure atrophy and haemorrhage; lymphoid hyperplasia of the gastro-intestinal tract and of the bladder; possibly early lesions of leucaemia in the heart; early stages of acute fibrinous pneumonia due to bacteria, with commencing disseminated necrosis and anthracosis; osteo-chondro-sarcoma in the mammary gland; gastric ulcers; few tapeworms; marked relative lymphocytosis, marked neutrophile leucopenia.

Summary.

The dominating cell in the lymphoid tissue, liver, bonemarrow and spleen, is one which has the structure of the haemocytoblast of the monophyletists, except possibly in the case of some of the cells in the spleen, its structure conforms more to that of the lymphoblast of the polyphyletists than to their myeloblast. That no differentiation of this cell into granulocytes is taking place to any extent is proved by the very marked neutrophile leucopenia, and further by the fact that more mature forms corresponding to the medium-sized lymphocytes are present in smears from the various organs examined. This indicates that differentiation is occurring towards the lymphocytes. As the total number of lymphocytes is normal or perhaps slightly increased the conclusion, that differentiation into lymphocytes is taking place, would appear to be justified. The hyperplasia involves primarily the lymphoid tissues of the body, but the bonemarrow and the liver also show marked lesions of lymphoid hyperplasia. The only organ other than the bonemarrow, in which tissue, suggestive of myeloid tissue, is found, is the spleen. Where such a generalized enlargement of lymphatic glands occurs throughout the body, it is necessary to differentiate and exclude malignant tumours, such as the lymphosarcomata. Many authors, Hutyra and Marek, Joest, Jakob, Naegeli, Kitt, MacCallum and others, emphasize the importance of this. The question arises whether the lesions described could have developed metastatically from the Osteo-chondro-sarcoma. The evidence against this is: this tumour is not a very malignant type; the sarcoma cells are spindle cells and should not metastasize as a large or mixed cell sarcoma; the lesions are peculiarly confined to a perivascular situation and are not infiltrative in their growth. The symmetrical involvement of the lymphoid tissues also speaks against malignancy.

All the available evidence favours a diagnosis of lymphatic aleucaemia, or lymphatic pseudo-leucaemia.

Conclusions.

A case of lymphatic aleucaemia is described in a bitch. The lymphoid hyperplasia involves the lymphatic glands throughout the body, the bonemarrow, liver, spleen, gastro-intestinal tract and the bladder. There is a very marked neutrophile leucopenia, a relative

lymphocytosis and evidence of compensatory myeloid metaplasia in the spleen. The latter explains the mild anaemia in spite of the severe destruction of bonemarrow that had occurred.

Acknowledgement.

To Miss Park of Gezina, District Pretoria, our sincere thanks are due for being good enough to make it possible for us to undertake this post-mortem and pathological study.

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Cystic Osteo-Chondroma of the Testicle in a Stallion.

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Subject: Brown Clydesdale stallion, number D.O.B. 19535, two years old, height 15.0 hands.

The stallion arrived at Onderstepoort on 20th May, 1929, to be treated for synovitis chronica serosa of the tendon sheaths of the M. flexor halucis longus. He was normally developed, showing the typical appearance of a two year old stallion. The horse developed symptoms of Nuttalliosis (*Nuttallia equi*) on the 8th June, 1929, and died on the 14th June, 1929.

A post-mortem examination showed that the right testicle was of normal shape, but small and abnormally soft. The left testicle was ovoid, larger than normal, for the most part very hard, with an uneven surface; size 11 by 9 by 6 cm., weight 0.35 Kg.

On section the right testicle was soft, and flabby. The left testicle was cut with difficulty. It contained seven cysts, more or less oval in shape, varying in size from 1 cm. to 2.6 cm. in length and from .4 cm. to 1.6 cm. in width (Fig. 1). The cavities were filled with a transparent viscid fluid. The walls of the cysts were mostly cartilaginous in nature, but in parts isolated areas resembling bone were discernible. The remaining portion of the testicle showed reddish-brown irregular areas of testicular tissue, which was softer than normal. There was also much yellowish tissue, which showed a lobulated appearance, resembling fat. (Fig. 1 shows a section through the long axis of the left testicle.)

On histological examination the walls of the cystic cavities showed white fibro-cartilage and here and there areas of bone formation (Figs. 2 and 3). The yellowish tissue showed fatty areas surrounded by fibrous septa. The reddish-brown tissue was testicular tissue in which no spermatogenesis could be observed. The seminiferous tubules were surrounded by loose thick bands of fibrous tissue.

Diagnosis: Cystic osteo-chondroma.

Fig. 1.
Photograph of a coloured drawing of a section made through the testicle.

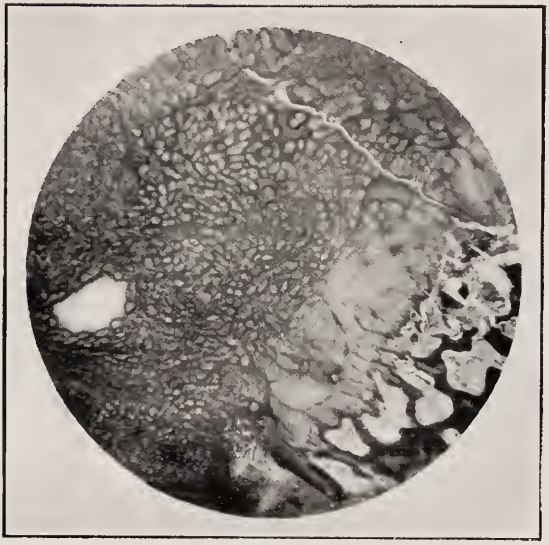

Fig. 2.

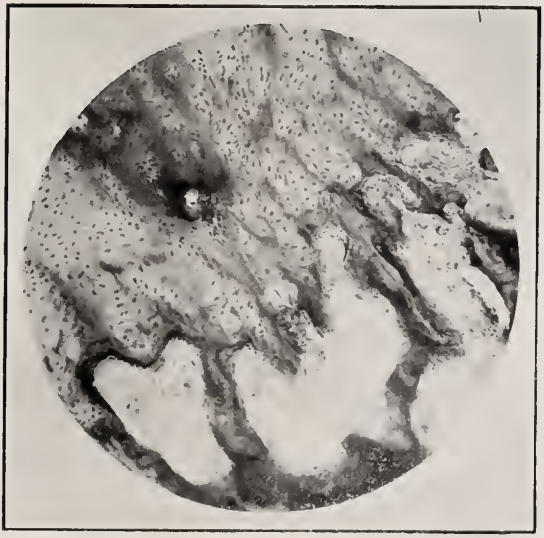

Fig. 3.
Sections through the tumour showing cartilage and bone formation.
2 × 30. 3 × 105.

Section VII.

Gland Grafting.

J. Quinlan, G. S. Maré, and L. L. Roux — Gland-Grafting in Merino Sheep. Preliminary Observations on its Influence: (A) on Body-development, Wool-production, and Progeny. (B) On Senility.

Gland Grafting in Merino Sheep.

Preliminary Observations on its Influence:

(A) On body-development, wool-production and progeny.

(B) On senility.

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G. S. MARÉ, B.Sc. Agric (S.A.) and

L. L. ROUX, M.Sc. (Ill.).

Since 1924 investigations have been carried out at this Institution to ascertain the possibility of increasing the skeletal development and, consequently, the production of mutton and wool in sheep.

The experiments have been based upon the publications of Steinach (1924), Ancel and Bouin (1924), (1927), and Voronoff (1924), (1928) namely, that by increasing the hormonic secretion of the male gonads there is rejuvenation in animals suffering from senile decay. Voronoff further claims to have produced increase in size, mutton and wool-production in grafted rams. He further states that this increase has been transmitted to their progeny. Steinach, and Ancel and Bouin maintain that ligature or section of the *vasa deferentia* causes atrophy of the reproductive cells of the male gonads. As a consequence, there is increase in the interstitial cells of the testicle, the internal secretion of which is responsible for the secondary sexual characteristics in the male. Quinlan (1928), however, pointed out that spermatogenesis was still apparent in sections of the testicle of a vasectomized sheep 2½ years after the operation. Humphrey (1926) says that vasectomy did not produce any disturbance of spermatogenesis or cause hypertrophy of the interstitial cells in a goat 18 months after the operation. Moore (1926) has also shown that ligation of the *vasa deferentia* does not cause aspermatogenesis unless there is malposition of the testis, neither has hypertrophy of the interstitial cells been observed. Lipschutz (1924) states that 1 per cent. of the testicular tissue is sufficient to maintain all the secondary sexual characteristics. Should this observation be correct, it appears unlikely that the hypertrophy claimed by Steinach, and Ancel and Bouin has any significance.

Quinlan (1928) published the results of his experiments on vasectomized sheep. He indicated that there were certain economic aspects in vasectomy as compared with ordinary castration inasmuch as it allows greater skeletal development, thereby providing for more mutton and wool. There were, however, disadvantages to the farmer,

as vasectomized sheep develop the secondary sexual characteristics of rams. Quinlan considers that vasectomy in immature ram lambs produces results not by increase in the interstitial tissue, as maintained by Steinach, but that the operated individuals develop normally as rams. The operation simply renders them sterile. It is possible that vaso-ligation or vaso-section may produce rejuvenation in humans suffering from senility, as maintained by Steinach. Such rejuvenation, however, would obviously be of little practical value in the lower animals, since it is associated with sterility.

Within more recent years, attempts have been made to ascertain the results of grafting lambs with sections of ovary and testicle, according to the methods of Voronoff.

OBJECT OF THE EXPERIMENTS.

The objects of these experiments were as follows:—

(1) To ascertain the effects of grafting the ovary of mature ewes, at two to four years, on young immature ewes at the age of 6 to 8 months.

A. On skeletal development and mutton-production.
B. On wool-production.
C. On the progeny.
D. On maturity and the oestrous cycle.

(2) To ascertain the effects of grafting sections of testicle taken from a mature ram at two years, on young immature rams at the age of 8 to 10 months.

A. On skeletal development and mutton-production.
B. On wool-production.
C. On the progeny.

(3) To ascertain the effects of mating a grafted ram with grafted ewes on their progeny, as regards skeletal development, mutton and wool-production.

(4) To ascertain the results of mating a grafted ram with non-grafted ewes on their progeny, as regards skeletal development, mutton and wool-production.

(5) To ascertain the effects of grafting testicle from a young healthy mature ram on a ram showing marked senile decay.

THE OPERATION.

Testicle Grafting in Rams.

The rams to be operated upon and the donors are confined to the stall, and put on to a ration of bran and succulent green food for a week prior to operation. For 24 hours before the operation solid food is withheld, but water is allowed up to the 12th hour before operating.

During the day previous to the operation the scrotum, caudal part of the hypogastrium and the medial aspect of both thighs are clipped of wool, washed with soap and warm water and shaved. The

scrotum is then washed off with ether, enclosed in sterile gauze and a suspensory bandage put on. The donor and the patient (recipient) are treated in the same way.

Two tables are used in the operation theatre, one for the donor and another for the receptors.

Deep general anaesthesia is practised. The method previously described by de Kock and Quinlan (1926), as adopted in splenectomy of sheep, has been used with entire success. The sheep is accurately weighed and receives an intravenous injection of 10 per cent. chloral hydrate in normal saline. The injection is carried out into the jugular vein. The dose used varies somewhat according to the age, length of starvation, and the condition of the patient. A good conditioned animal stands a slightly larger dose than a badly conditioned sheep. The dose given accordingly varies from 1.25 to 2.0 grams per 20 Kg. body-weight. This dose is sufficient to produce deep anaesthesia for half to three-quarters of an hour. In case anaesthesia is not sufficiently deep, a few drops of ether are used to complete it.

The donor is first anaesthetised by an injection of chloral hydrate somewhat larger than that usually used for the recipients.

The following summary shows the average dose of chloral hydrate, the average weight of the sheep, and the average duration of anaesthesia in 50 sheep anaesthetised by this method: —

Weight of Sheep.	*Chloral hydrate.*	*Duration of anaesthesia*
31·5 Kg.	2·72 grams.	41·5 minutes.

The donor is kept completely anaesthetised for about 2 hours and yields grafts for four sheep. Anaesthesia of the donor having been completed, the recipient is anaesthetised. The suspensory protective bandage is then removed, and the skin of the scrotum washed off with ether. The abdomen and hind limbs are then covered with sterile calico cloths. A window is made over the scrotum, and the sac and testicles pulled through. The patient is then placed in the lateral position and the scrotum laid on sterile muslin.

The donor is then operated upon. The skin and dartos are cut through so that the parietal layer of the tunica vaginalis communis is exposed. It is dissected free from the overlying dartos and the testicle covered by the tunica vaginalis communis withdrawn. An incision about 10 cm. in length is made through the tunica exposing the testicle. The borders of the cut in the tunica are taken up in artery forceps, so as to prevent the testicle protruding. The spermatic cord is caught loosely in an artery forceps so as to control haemorrhage when the testicle is cut into. Having covered the scrotum over with sterile gauze, the donor is left until the receptor is prepared for grafting.

The receptor is now prepared to receive the graft. The preliminary aseptic precautions have already been carried out as has been done in the case of the donor. An incision about 8 cm. in length is made through the skin on the lateral aspect of the scrotum. The tunica dartos is not yet incised. The skin is raised from the dartos all round the incision for a distance of about 1.5 cm. The borders are then caught up in forceps. The dartos is incised along the entire length of the skin wound. It is raised from the parietal layer of

the tunica vaginalis communis and a pocket made with the handle of a scalpel between the tunica and the dartos to receive the graft. An assistant holds the testicle in position while the surface of the tunica vaginalis communis is scraped. This scraping is done with a pointed instrument and must be done carefully. Experimental operations previously carried out proved that scraping must be very thorough, otherwise the piece of tissue inserted does not become vascularized, but sloughs away as a sterile slough in 3 to 5 weeks' time.

The method employed in these experiments has been extravaginal. Intravaginal grafting has not been too successful in our hands in sheep. The intravaginal cavity is potential and when grafts of reasonable size have been placed within it difficulty has been experienced in suturing the parietal tunica without tearing. There was always a marked fibrous periorchitis remaining, even though the scrotal wound healed *per primam intentionem*. In the case of intravaginal grafting, failure of vascularisation and sloughing of the graft also appears to be more frequent than with the extravaginal method.

The wound is now covered with sterile gauze while the operator removes the pieces of testicle to be grafted from the donor. Sections, 4 by 1.5 by 1 cm., are removed with the forceps and scalpel, leaving the tunica vaginalis visceralis attached. Larger sections have been tried, but these failed to become vascularised and became necrosed, while smaller sections appeared to undergo rapid absorption in a few weeks' time. The section is immediately placed in position in the pocket made in the recipient, the glandular surface lying on the prepared tunica. It is then sutured with No. 00 catgut, by placing a suture at either extremity and one on either side. In case any of the testicular tissue bulges between the ligatures, one or more further sutures are inserted until the graft lies nicely attached to the tunica. It appears necessary to take out the graft from the donor with the tunica vaginalis propria attached, as the tissue is soft and it is almost impossible to suture it without the tunica; the sutures cut through easily and the graft drops off. The tunica dartos is now sutured over the graft by a continuous catgut suture. The skin wound is closed by interrupted silk sutures or skin clips. It is then sealed with autosept. The graft is attached to the other testicle in the same way. The scrotum is covered with sterile gauze and enclosed in a suspensory bandage. Healing in the experimental rams took place *per primam intentionem* without swelling or any constitutional disturbance. The skin sutures are removed on the 5th to the 7th day. Owing to the small size of the testicle at the time of operation, only one graft of the size mentioned was inserted on either side. In the old ram treated for senility two such pieces were inserted on either side.

The donor supplies grafts for 4 rams, two being grafted from each testicle. When the operation is completed the donor is castrated, but the grafts are taken while the testicles are *in situ*. The donor is usually under anaesthesia for 2 to 2½ hours and afterwards shows no ill-effects.

Ovary Grafting in Ewes.

The ewes are prepared in the same way as the rams. They are confined to the stall for a week, and are given a ration of green food, bran, a little maize meal and oats. Twenty-four hours before operation food is withheld, but water is given up to the twelfth hour. The day preceding the operation the region of the left flank is shorn clean of wool and shaved. It is then washed off with ether. The donors and receptors are similarly prepared.

One donor and two recipients are brought to the operation theatre at the same time. The donor and one receptor are anaesthetised. The region of the left flank is covered over with sterile calico, in which a window has been made.

The recipient is then prepared for the graft. An incision is made through the skin of the left flank. The cloths are attached to the lips of the incision by forceps. A pocket is then made to receive the grafts either between the m. obliquus interus and the m. transversus abdominis, or between the m. tensor fascia lata and the m. obliquus externus. The pocket is made by dissection with the handle of the scalpel.

The surface of the muscular sheath is scratched with a sharp instrument until there is an exudation of blood all over the area to which the graft is to be attached. The wound is then covered with a sterile cloth. The operator's attention is now transferred to the donor.

A laparotomy is performed in the left flank and one ovary removed. The ovary is incised deeply along its length and the cut surface immediately applied to the prepared surface of the muscle of the recipient. It is then sutured in position by No. 00 catgut, using 2 or 3 sutures. The muscular wound is also sutured with a continuous catgut suture. The skin is closed with interrupted silk sutures and the wound sealed with autosept. Healing in every case took place *per primam intentionem.* The stitches were removed on the 6th to 7th day.

Two sheep are grafted from each donor. These operations were performed during the seasonal period of ovarian activity of sheep in this country. Therefore, the grafted ovaries were in all stages found during the ovarian cycle, from the fully developed corpus luteum to the fully developed Graafian follicle.

Local Results of the Operation.

In the rams the grafts could be distinctly felt, apparently surrounded by connective tissue, attached to the tunica vaginalis communis for several months. After a period of 2½ to 3 months they began to atrophy slowly, and at the end of 8 to 12 months no trace of them could be felt.

The fate of the grafted tissue is interesting, since if permanent results are to be obtained, one would expect that after vascularisation the histological appearance of normal testicular tissue should be perceptible, or at least that the interstitial cells should be recognisable. It appears that the grafted tissue entirely vanishes within a period of 12 months. On a careful dissection of the site of the attachment,

no trace of it could be found. It is quickly surrounded by connective tissue, and can be felt through the skin. It begins to decrease in size about the second to the third month, and has entirely disappeared at 12 months; in some cases even at 6 months.

Histological examination of grafts sectioned 110 days after operation has shown the centre containing hyalinised remnants of the seminiferous tubules, the lumena of which are filled with neutrophiles (Figs. 1 and 2). Interspersed between the remnants of the tubules, and also in a deep zone surrounding this area, there is an accumulation of neutrophiles and round cells (Figs. 1 and 2). Surrounding this latter area is a zone containing epithelioid cells, round cells, fibroblasts and foreign-body giant cells (Figs. 3 and 4). Outside this zone there is a zone of fibrous tissue.

The graft seems to act as a foreign body. The only recognisable testicular tissue, namely the hyalinised remnants of seminiferous tubules, is dead and in a process of absorption. The cells observed are those which occur in lesions showing the ordinary reaction seen in the resorption of foreign bodies.

Voronoff (1924) submitted specimens of grafts for histological examination to Dr. Retterer, Professor and Fellow of the Faculté de Medicine of Paris. These grafts were taken from he-goats and rams month by month after they had been grafted by Voronoff. The experiments, according to Voronoff, extended over a period of nearly two years. The grafts during the first month showed a certain amount of disintegration of the epithelial lining of the seminiferous tubules, while the interstitial tissue seemed to spread out. At the end of a year the cells lining the tubules had increased to such an extent as to fill up the lumena, converting them into cords. It is stated " . . . a testicle taken from a six months old ram and implanted into the scrotum of a senile ram on 7th May, 1918, has continued progressively to develop, and has passed from the prepuberal stage to that of spermatogenesis."

At the time of grafting, a section of this testicle examined by Dr. Retterer was described as in the pre-spermatic stage; that the tubules measured 15 millimetres; and that they were lined by several layers of epithelial cells without spermatids or spermatozoa. Fourteen months after grafting sections of the graft showed " the tubules at periphery of these portions contained small nuclei and flattened oval filaments, having all the morphological and staining characters of the heads of spermatozoa."

These observations satisfied Voronoff that the implanted portions of testicle had lived and developed, and that they had not remained as inert foreign-bodies destined to gradual absorption by surrounding tissues. Consequently, the phenomena which had been observed in the grafted animals were considered to be the result of hormones secreted by the living graft, not the result of opotherapic absorption of the slowly perishing gland.

Referring to the duration of the grafted tissue in the host Voronoff recognises that it is not permanent, but he maintains that a second graft is quite as efficacious as the first. The good results of grafting have been maintained over at least 3 years. It appears

to be Voronoff's hypothesis that the influence of the graft is not only to rejuvenate the host by hormonic secretion, but actually to instil rejuvenation into the gonads; this rejuvenation of the gonads being maintained long after the graft has been absorbed, or at least long after any trace of it can be detected by examination of the operated site.

It is regrettable that a complete histological examination of the changes effected in the testicles of animals suffering from senility by grafting has not been published. Such an examination would have thrown much light on the question. It appears likely that any permanent change from impotency to potency would be associated with some histological rejuvenation as well as physiological rejuvenation in the grafted host. It would, therefore, appear to be an excellent method of studying the duration of the apparent physiological improvement described by Voronoff in his grafted animals.

The histological examination of the testicle of a senile ram, grafted 3 times, namely on 16-9-27, 12-6-28 and 8-8-29, showed that there was a condition of aspermatogenesis. Sections taken from both testicles showed some tubules with an entire absence of seminal epithelium. In other tubules the seminal epithelium had become detached from the tunica propria and existed as a distintegrated cast of the lumen. In other tubules again, the cells of Sertoli and spermatogonia were recognizable; the cells derived from the latter were degenerated. There were no spermatocytes, spermatids, or spermatozoa. No increase could be recognized in the interstitial cells or connective tissue. (Figs. 5 and 6.)

Recent work of Velu and Balozet (1929) on the fate of testicular grafts has shown that in all cases there is resorption of the graft. The rapidity of resorption depends on whether the grafts are homoplastic or heteroplastic. The heteroplastic grafts are more quickly resorbed. Histological examination of a 7-days-old graft showed that the centre was necrosed. The periphery of the necrosed area was well defined. At the junction there was a large accumulation of embryonic cells in the intertubular spaces with some leucocytes. Outside this zone there was a zone of connective tissue and tubules, with tubular resorption at the periphery. Some of the tubules still had an intact membrane, but did not contain any living cells. Here and there spermatozoids were still recognizable. Occasional tubules were seen containing certain cells of epithelial appearance with large nucleus and nucleolus, like cells of Sertoli. These living remaining cells were very rare and were seen only in the periphery where the nutritive plasma of the host was able to penetrate first.

Velu and Balozet consider these cells epithelioid in nature, as they believe they are derived from the embryonic cells of the receptor. They say that these cells are not seen before the seventh day after the operation. They appear to be elements originating from the host and are directly concerned with the resorption and destruction of the graft, somewhat after the type of cells seen in tubercular lesions. Further they are not seen in sections taken on the 14th day after grafting.

The authors conclude that, in spite of the presence of these epithelioid cells, death of the graft takes place as a rule; that the

apparent revival of the graft is merely due to a phenomenon of substitution by migrating cells from the host into and along the interstitial tissue of the graft in order to absorb it. They maintain that it really is not a graft in the strict histological sense, but rather an insertion or implantation.

The situation of the grafted ovary in one of the ewes was carefully examined 5 months after the operation. No trace of the grafted ovary could be found and the histological examination of the connective tissue from the site failed to reveal any elements of ovarian tissue. It appeared to have been completely absorbed.

In discussing the results of grafting rams before puberty, or at the beginning of puberty, that is immature rams, Velu and Balozet (1929) state that there may be a temporary increase in body-weight during the three months following the operation. After 3 months the development of the grafted rams was slower than of the controls. The authors indicate that the results were more marked when very young ram lambs were grafted. There was little or no increase in the weight of wool produced.

They conclude that grafting does not appear to have any beneficial influence either on the body-weight or the wool-production. They even suggest a deteriorating influence inasmuch as it may cause temporary forced development during the period of immaturity, thereby reducing the duration of subsequent development and eventual size. At the present time they suggest that the only solution to improvement in flocks is through judicious selection and breeding.

Petit in discussing the experimental results of Velu and Balozet, appears to think that the failure of these authors to substantiate the data of Voronoff is due to insufficiency of material. He draws attention to the report of the International Commission to Algeria, of which M. Carouyeau and M. Moussu were delegates, as conveying more reliable evidence on the work than the data of Velu and Balozet. However, he admits that the results of Voronoff's experiments do not entirely stand scientific criticism, as he sees the necessity for a repetition of this work at the National School of Agriculture.

Marshall, Crew, Walton and Miller, the English delegates on the International Commission to Algeria, have published a critical report on their observations. Their conclusions are concise, so that it is justifiable to quote them in detail. They state:—

> "The claim of Dr. Voronoff to effect rejuvenation of the aged and decrepit male by the operation of testis grafting is possibly justifiable. The evidence is, however, not based upon critical experimentation. Data on the duration of the graft and its effect upon the fertility of the operated animal are at present insufficient to warrant an estimate of the economic value. It is an open question whether this method, even if or when its merits have been fully demonstrated, can become of any considerable importance in Britain. Alternative methods of extending the usefulness of valuable sires are available and might be adopted with equal advantage.
>
> The claim of Dr. Voronoff to increase the body-weight and wool-clip of the ram by the operation of testis grafting applied to the immature animal is supported by the figures submitted

and by the sheep shown to the delegates, but the conditions under which the experiments have been conducted, the inadequacy of the data submitted, and the methods of presentation of the data, exclude any possibility of forming a critical opinion. The direct economic advantages of the operation are negligible.

The claim of Dr. Voronoff that the improved characteristics shown by the grafted rams are transmitted to the offspring (males, females and castrates) involves the acceptance of a principle of heredity (the inheritance of acquired characteristics) which has not been substantiated in the realm of science and could only be so by appeal to the most critical and extensive research. These requisites have not been fulfilled in Dr. Voronoff's experiments. The results are capable of other explanations based upon established principles of heredity, for example, chance isolation of superior hereditary strains, aided possibly by (unconscious) selection.

Assuming, however, the validity of the claim, the economic value of increased body-weight and wool-clip would have to be considered not only in relation to climatic conditions and economic demands but to the increased cost of upkeep of the animals. The value of the method must also be compared with existing methods of improvement, for example, pedigree-breeding and selection, importation of superior stock, progeny tests, and the like."

Kronacher, Henkels, Schäper and Kliesch (1929) have recently published the results of observations carried out on a male goat operated upon strictly according to technique of Voronoff. Their observations were limited to one operated animal as compared with one male and one castrated control, but they were carried through with the utmost accuracy, and accordingly are of great value. In their conclusions the authors state that their results regarding skeletal development, body-weight and sexual characteristics, do not substantiate the claims of Voronoff. Photographs of the experimental and control animals clearly indicate that there is no significant difference between them.

The authors after a period of one year made a histological examination of the transplanted sections of testicle, and found marked degeneration with invasion with connective tissue. There are several excellent photomicrographs to illustrate the histological changes which have taken place in the grafted tissue.

THE EXPERIMENTS.

A. GLAND GRAFTING ON BODY-DEVELOPMENT, WOOL-PRODUCTION AND PROGENY.

The experiment was begun in November, 1927. It was carried out on a flock of pure-bred Merino sheep of a very good class, and selection was made from lambs born in the previous autumn and early winter. The ages varied from six to eight months. The ewes were chosen from the Grootfontein School of Agriculture's large selected flock. The two groups, the grafted and the non-grafted, were

made as uniform as possible with respect to carcase and type of fleece. When a sheep was chosen for grafting a sheep of a similar type was put in the control flock.

The four grafted and the four control rams were selected in the same way. In each group there were two Wanganellas and two Tasmanians. Further, there was a close relationship from the sires' side in the sheep of each group.

By this method of selection it was intended to eliminate as far as possible the tendency to individual variation of body-weight and weight of wool due to inherent differences.

The sheep after the operation were kept under observation at Onderstepoort until the 15th June, 1928, to observe the onset of oestrus with a view to ascertain whether the operated ewes would show earlier maturity. They were tested for oestrus by using two vasectomised rams. The 25 grafted sheep and the 25 controls showed oestrus during the month of January, 1928, when they were about 9 to 10 months old. This is what is usually experienced under South African conditions. Lambs born in the autumn and early winter show oestrus the following summer at the age of nine to ten months, if maintained in good condition. It has been pointed out by Küpfer that the Merino sheep in South Africa has a seasonal oestrous cycle, the period of activity of the ovary being confined to late summer and early winter. Küpfer's limited observations on the Merino sheep showed a period of ovarian inactivity, which was interrupted at the commencement of January. It is well known, however, that in a good season, when green food is plentiful, the period of activity of the ovarian cycle begins in October and early November.

During the period of ovarian activity in the following season, 1928-29, observations were also carried out on the occurrence of oestrus. In this connection a comparison was made between the grafted ewes and four groups of non-grafted ewes, each approximately containing the same number as the grafted group.

The following table indicates the occurrence of oestrus: —

TABLE 1.

Group.	Numbers showing Oestrus.				No Oestrus.
	Dec.	Jan.	Feb.	March.	
A.—23 Sheep (even numbers 18676–18724, grafted)	3	15	2	—	3
CONTROLS.					
B.—25 Sheep (odd numbers 18677–18725, non-grafted)	4	17	1	—	3
C.—25 Sheep (1–25 G.S.A., non-grafted)	3	12	6	1	3
D.—25 Sheep (3655 and 26–49 G.S.A., non-grafted)	5	13	4	—	3
E.—25 Sheep (50–74 G.S.A., non-grafted)	3	17	3	—	2

The following table indicates the duration of the interoestrous period in the five groups of sheep, namely, the grafted group and the

four groups of non-grafted controls. The sheep were tested during the mating season by using vasectomised rams as " teasers ":—

TABLE 2.

Periodicity of Oestrus.

Group.	Interval in Days.															
	16	17	18	19	20	21	22	23	24	32	33	34	35	36	37	39
A.—Even Nos. 18676–18724 (grafted).....	2	3	2	1	1	1	.	1	.	2	.	1	1	1	.	.
B.—Odd Nos. 18677–18725 (non-grafted).....	2	3	5	.	.	.	1	.	1	1	.	.	.	1	.	.
C.—G.S.A. Ewes, 1–25 (non-grafted).....	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
D.—G.S.A. Ewes, 3655 and 26–49 (non-grafted)	.	1	.	2	.	.	.	.	.	.	.	.	1	.	1	1
E.—G.S.A. Ewes, 50–74 (non-grafted).....	3	2	1	.	.	.	.	.	.	.	2	2	4	1	.	.
TOTALS.....	7	9	8	3	2	1	1	1	1	3	2	3	6	3	1	1

NOTE.—These figures are compiled from the numbers of sheep which returned to service.

It will be obvious from the above table that the interovulation period in the sheep varies between 16 and 24 days. The majority showed an interovulation period of 16-18 days. The occurrence of interovulation periods ranging from 32 to 39 days indicates that the ovarian cycle in some cases may be double the normal period, or that " silent oestrus " also occurs in the sheep as is observed in bovines. It is unlikely that normal oestrus occurred, and was missed as the observations were carried out daily with the utmost care. This would not quite correspond with Küpfer's observations on the periodicity of oestrus in the sheep. He states that oestrus occurs regularly at intervals of 18 days during the period of ovarian activity.

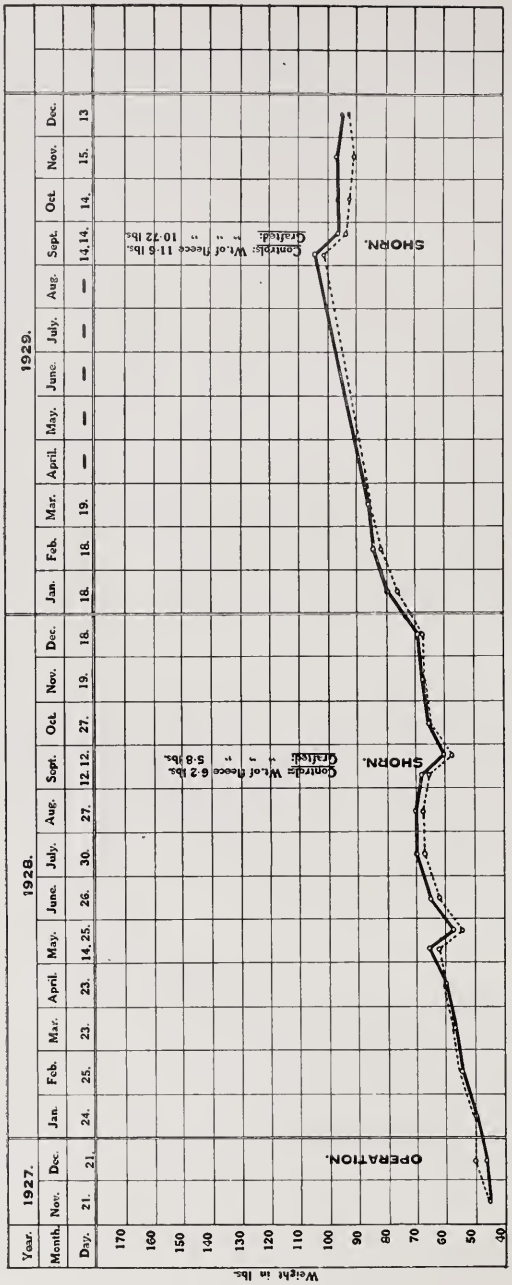
While under observation for two seasons, no difference in ovarian activity could be observed between the grafted and non-grafted ewes.

The two groups, A and B, were transferred to the School of Agriculture, Middelburg, Cape Province, on the 15th June, 1928, where they have since been running on the excellent sheep farm maintained by that institution.

The grafted and control ewes were weighed, after starvation for fifteen hours, at monthly intervals over a period covering two years, with the exception of a period of six months from March to September, 1929, when they were close to the end of gestation or suckling young lambs. During this period individual weights were considered to be of little value, due to exceptional variations existing in the groups.

There was little variation in the average body-weight of either group, as shown in the following graph. In fact, any difference that is shown, may be considered quite insignificant:—

Graph No 1.
Ewes.
AVERAGE WEIGHT.



The sheep were shorn at 12 months interval in September, 1928, and 1929. The following table indicates the shearing analysis for the two years:—

TABLE 3.

Wool-Yields—Ewes.

Group.	1928.			1929.		
	Shirted.	Fleeces.	Skirtings.	Skirted.	Fleeces.	Skirtings.
	Range.	Average.	Average.	Range.	Average.	Average.
	lb.	lb.	lb.	lb.	lb.	lb.
A.—Grafted.....	1·9–4·6	3·4	2·4	4·5–10·1	6·8	3·9
CONTROLS.						
B.—Non-grafted...	2·2–5·2	3·9	2·3	3·9–10·2	7·2	4·4
C.—Non-grafted...	—	5·9	2·5	—	7·2	4·2
D.—Non-grafted...	—	5·6	2·7	—	6·6	3·9
E.—Non-grafted...		5·8	2·2	—	7·3	4·1

NOTE.—It is noticeable that Groups A and B, which were transferred to Onderstepoort, out of their natural Karroo environment for 6 months in 1927-1928, shore less wool at the 1928 shearing that Groups C, D and E, which were not transferred. However, their recovery in the following year is apparent.

The following table indicates the classes of fleeces produced by the respective groups during the two seasons:—

TABLE 4.

Group.	E.S.C.		S.S.C.		C.		C.C.		TOTAL.	
	1928.	1929.	1928.	1929.	1928.	1929.	1928.	1929.	1928.	1929.
A.—Grafted.....	9	19	4	4	10	—	—	—	23*	23*
CONTROLS.										
B.—Non-grafted†	14	14	2	10	6	1	3	—	25	25*
C.—Non-grafted†	15	17	5	4	4	2	1	—	25	23*
D.—Non-grafted†	14	19	5	5	6	—	—	—	25	24
E.—Non-grafted.	11	19	6	4	8	—	—	2	25	25

* The decrease in numbers from the original 25 sheep in each group is explained by the fact that a sheep had died, or was sick at the time of shearing.

† The number of fleeces in the columns for 1929 in the three latter groups have been computed from the weights of wool given by each individual lot in these classes. The total weight in each class rather than the number of fleeces has been taken.

NOTE.—The following indicates the meaning of the abbreviations used in the above wool-classing standards:—

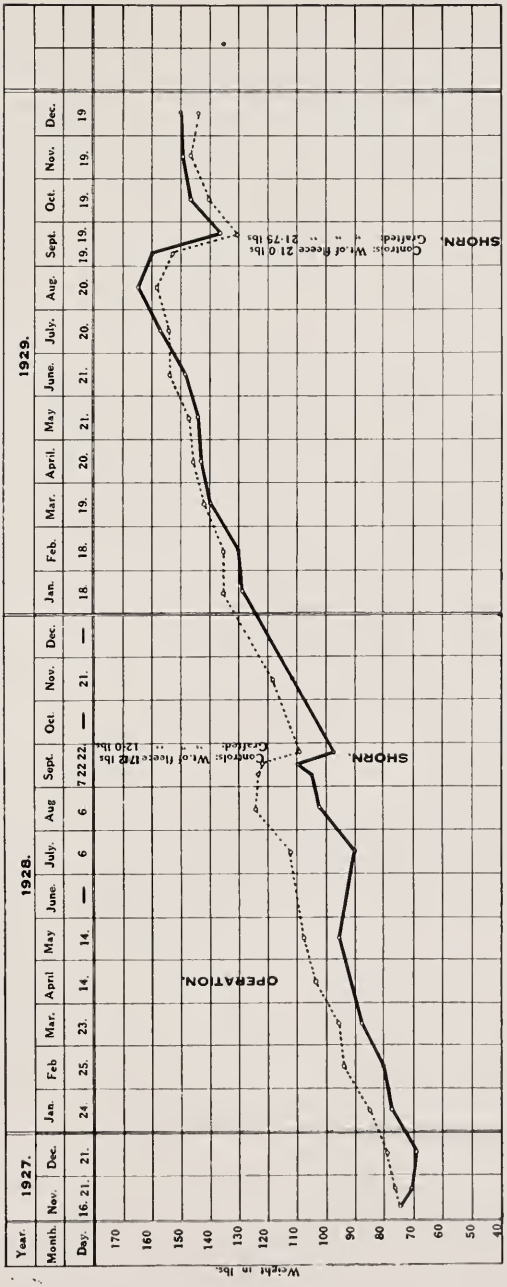
E.S.C.: Above average 64s quality, $2\frac{1}{4}$ inches and over in length.
S.S.C.: Average 60s/64s quality (not under), $2\frac{1}{4}$ inches and over in length.
C.: Not under $2\frac{1}{4}$ inches in length.
C.C.: Not under $1\frac{3}{4}$ inches in length.

The grafted and control rams were weighed at monthly intervals over a period covering two years. The four rams for grafting were transferred to Onderstepoort on the 16th November, 1927; at that time they were the same weight as the four control rams. It will be noted in the following graph that the grafted rams did not again attain the weight of the non-grafted rams until March, 1929. The actual difference can be explained by the change in environment. The grafted rams were kept at Onderstepoort for the period from November, 1927, to June, 1928. The change from the Karroo veld to the Transvaal, had apparently influenced them somewhat. They were stall fed on lucerne hay, crushed oats and maize, and were given limited grazing on Kikuyu grass. It was impossible to let them run on the veld owing to the danger of heartwater infection. Further, it took them some months to catch up to the control rams again, even when they were placed in their original Karroo conditions.

It must be explained that the grafted rams were always kept in excellent condition, but owing to the changed environment, or to the operation, they did not develop as quickly as the controls. For the past nine months, namely March to December, 1929, the range of the average body-weight between the two groups is negligible.

It would have been more convincing if the two groups had been treated in a similar manner as regards environment, as was done with the ewes. This omission is now being corrected in a further series of grafting experiments.

Graph No 2.
Rams.
AVERAGE WEIGHT.



The rams were shorn at 12 months interval in September, 1928, and 1929; at the first shearing there was a difference of 5.12 lb. of wool per sheep in favour of the controls. This can also be explained as being due to the different environmental conditions. At the last shearing there was a difference of .75 lb. of wool per sheep in favour of the grafted group.

The following table indicates the shearing analysis for the two years:—

TABLE 5.

Wool-Yields—Rams.

Ram.	1928.		1929.		
	Fleece-weight.	Class.	Fleece-weight.	Class.	Spinning Count (average).
	lb.		lb.		
Grafted Rams.					
W. 20	11·5	S.S.C.	22·0	S.S.C.	60's
*W. 30	14·0	S.S.C.	24·5	S.S.C.	60's
T. 402	13·0	S.S.C.	22·0	S.S.C.	60's
*T. 413	9·5	S.S.C.	18·5	E.S.C.	70's
Control Rams.					
W. 9	16·0	S.S.C.	20·0	E.S.C.	64's +
*W. 31	18·5	S.S.C.	22·5	S.S.C.	60's −
W. 49	15·5	E.S.C.	18·5	E.S.C.	64's +
*T. 417	18·5	S.S.C.	23·0	S.S.C.	64's

* These rams were used in the experiment.

NOTE.—The following indicates the meaning of the abbreviations used in the above wool-classing standards:—

E.S.C.: Above average 64's quality, $2\frac{3}{4}$ inches and over in length.

S.S.C.: Average 60's/64's quality (not under), $2\frac{3}{4}$ inches and over in length.

In December, 1928, January and February, 1929, the sheep were mated to observe the influence of grafting on the progeny.

The system of mating was as follows:—

1. Grafted ram W. 30 x 23 grafted ewes (Group A).
2. Grafted ram W. 30 x 25 non-grafted ewes (Group B).
3. Grafted ram T. 413 x 25 non-grafted ewes (Group C).
4. Non-grafted ram T. 417 x 25 non-grafted ewes (Group D).
5. Non-grafted ram W. 31 x 25 non-grafted ewes (Group E).

It must be noted that the same grafted ram W. 30, was used on two groups of ewes—grafted and non-grafted. The above rams were used exclusively for these matings. The experimental rams not mentioned, were not used, but were kept in reserve in case of accidents.

In the continuation of the experiment this same system of mating is being employed.

The "handserving method" of mating has been adopted throughout. The ewes were tested with a vasectomised "teaser" and were allowed one service only at an oestral period.

The following table further indicates the system of mating, the details of service and the lambs born to each group:—

TABLE 6.

First lamb born 24th May, 1929. Last lamb born 31st July, 1929.

Ewes.—Group.	No.	Sire.	Ewes Served.				Ewes not Served.	Ewes Lambed.	Percentage Lambing.
			Once.	Twice.	Three times	Total.			
A.—Grafted.....	23	W. 30—Grafted.....	7 *(4)	11 (4)	2	20	3	8	34·8
B.—Non-grafted.....	25	W. 30—Grafted.....	9 (7)	12 (1)	1	22	3	8	32·0
C.—G.S.A., non-grafted....	25	T. 417—Non-grafted....	15 (12)	6 (5)	1	22	3	17	68·0
D.—G.S.A., non-grafted....	25	T. 413—Grafted.....	21 (21)	1 (1)	—	22	3	22	88·0
E.—G.S.A., non-grafted....	25	W. 31—Non-grafted....	9 (3)	13 (2)	1	23	2	5	20·0

* Figures in brackets indicate number of lambs born to the corresponding service.

The low percentage fertility is interesting. It has been observed in this country that when first season rams are mated with first season ewes, the lambing percentage is invariably low. The incidence of barrenness in yearling ewes as compared with older ewes, has been noted by Nichols (1926) in the British Isles.

The weight of the individual lambs was taken at birth, and monthly weighings were commenced in November, when the average ages in the groups ranged from 140 to 155 days.

The following table indicates particulars of interest of the lambs from the various groups of ewes: —

TABLE 7.

Particulars of Lambs Born to Groups of Experimental Sheep.

Sire.	Group.—Dams.	Weight at Birth.*		No.	On 3/10/29.			No.	On 11/11/29.				No.	On 11/12/29.			
					Age.		Weight of Wool Shorn. Average. lb.		Age.		Weight.			Age.		Weight.	
		Range lb.	Ave. lb.		Range. Days.	Ave.			Range. Days.	Ave. Days.	Range. lb.	Ave. lb.		Range. Days.	Ave. Days.	Range. lb.	Ave. lb.
W. 30—Grafted	Group A, even Nos. 18676–18724, grafted.....	7·7– 9·7	8·6	6	88–127	112	2·75	6	127–166	151	49·5–72·5	58·0	6	157–196	181	53·5–77·5	61·9
W. 30—Grafted	Group B, odd Nos. 18677–18725, non-grafted.....	7·9–11·0	10·1	7	107–125	115	2·30	7	146–164	154	56·5–68·5	61·5	7	176–194	184	50·0–71·5	62·8
T. 413—Grafted	Group C, 1–25, non-grafted.....	7·1–11·2	8·6	18	62·133	102	2·0	18	101–172	141	45·0–65·0	54·2	18	131–202	171	47·5–68·5	58·4
T. 417—Non-grafted	Group D, 3655 and 26–49, non-grafted.	4·5–10·9	8·4	12	90–133	109	2·2	12	125–172	148	43·0–67·0	56·7	12	155–202	178	47·5–74·0	60·3
W. 31—Non-grafted	Group E, 50–74, non-grafted.....	7·5–10·9	8·4	3	108–129	116	2·25	3	147–168	155	56·0–65·5	61·2	3	177–198	185	62·0–70·0	66·8

* Including lambs born dead or died shortly afterwards.

On consulting the above table, it will be observed that, with the exception of the lambs from Group B, the average weight at birth was practically identical. The high average in the case of Group B is due to three exceptionally heavy lambs out of a total of eight born. Any difference in subsequent weights, namely in November, may be ascribed to the fact that the average age of the lambs in the different groups varied considerably.

The lambs were shorn in October, 1929. The differences in average wool-production are not sufficient to justify the conclusion that there were any special characteristics transmitted to the offspring of the grafted animals.

Conclusions.

(1) Grafting ovaries into ewes and sections of testicle into rams has been successfully performed from a surgical standpoint under general anaesthesia.

(2) Ovary grafting in immature ewes does not exercise any beneficial influence on maturity, body-weight, wool-production, or progeny.

(3) Testicle grafting in immature rams does not exercise any beneficial influence on maturity, body-weight, wool-production or progeny.

(4) Mating grafted rams with grafted ewes has no influence on their progeny, judged by weight at birth, and at a range of age averaging about 157 to 196 days, nor has it indicated any advantage on the weight of lamb-fleeces shorn.

(5) The interovulation period in the merino sheep varies between 16 and 24 days, with an average of 17-18 days.

(6) In the 5 groups of first season ewes mated with first season rams, there was a low percentage of fertility, ranging from 20 to 88 per cent.

(7) Out of 123 first-season ewes, 14 or 11.4 per cent. failed to show oestrus when tested daily with vasectomised "teasers" over a period of 3 months—December to March.

B.—GLAND GRAFTING ON SENILITY.*

A valuable Wanganella ram No. 18342, aged 13 years, which had been imported from Australia, was submitted to the operation of grafting to observe its influence on senility. He was an ideal subject. The ram had been used as a stud sire at the School of Agriculture, Glen, Orange Free State, for a number of years. Within recent years he began to show marked signs of approaching senility, until for two seasons he was quite impotent. He exhibited no interest in ewes showing oestrus. He had little appetite and gradually lost condition. His fleece became loose and lost brightness. He was anaemic and somewhat hide-bound. He walked stiffly and could not rise unless assisted. He would lie for hours stretched out in his stall. When standing his head was held low with listless expression. There

* In this work Dr. Quinlan was assisted by Mr. G. Pfaff, B.Sc., M.R.C.V.S.

was incontinence of urine, which resulted in ulceration of the skin around the opening of the sheath. The ulceration, which had apparently existed for a considerable time, caused stenosis of the opening of the sheath, which resulted in partial retention of urine. The wool of the abdomen was always soiled with urine. The sheep had lost all his incisor teeth. His stifle joints crepitated on movement, apparently the result of arthritis sicca. The heart was apparently anatomically sound, but the pulse was weak. The lungs were normal. The stomachs and intestines were functioning normally. To summarize, his symptoms appeared to be in the last stages of senile decay, and that he had not many months to live was evident.

On the 16th September, 1927, the sheep was grafted with a section of testicle from a normal two-and-a-half years old ram. That the grafted testicle was normal was proved by histological examination. The size of the graft was 5 by 5 by 1 cm. It was removed from the donor with the visceral layer of the tunica vaginalis attached. The donor and the receptor were prepared, as already described. The receptor was anaesthetised by an intravenous injection of 4.5 grams of chloral hydrate in a 10 per cent. solution with normal saline, followed by 2 c.c. of chloroform as an inhalation. Complete anaesthesia lasted for an hour.

This operation was carried out in the left flank-region in the same way as the experimental ewes were grafted. The graft was placed in a pocket between the m. tensor fascia lata and the tendinous portion of the m. obliquus externus. The capsule of the tensor fascia lata was prepared by scarification. The glandular portion of the graft was placed on the prepared surface, and the graft fixed in position by six sutures, using No. 00 catgut. The skin wound was sutured with silk.

Recovery from the operation was uneventful; the wound healed *per primam intentionem*, the skin sutures being removed on the 8th day.

At the time of operation the ram weighed 126 lb., and carried about 10 months growth of wool, which was loose and lacked brightness. Two weeks after the operation there was a marked improvement in the general condition of the ram. He was clipped on the 11th October, but shore only 9 lb. of wool. The improvement continued until the 12th December, 1927, when he was sent back to the School of Agriculture, Glen, to be tested for potency.

As slight retention of urine in the sheath continued after the operation, although the ulceration healed with treatment, an operation for phimosis was carried out on the 12th October, under local anaesthesia, using tutocain. The operation wound healed rapidly.

While under observation here up to the 12th December, 1927, the sheep made an extraordinary recovery. He put on weight, showing an increase of 15 lb., and became more active. He fed well and showed interest in two ewes which were put in his " kraal " daily. However, he never copulated with them, although they should have been in oestrus as they were with him during the early part of the period of ovarian activity in sheep in this country.

While at the School of Agriculture, Glen, the improvement in the ram's condition was maintained for some months. He became pugnacious and would fight with younger rams so that he had to be mostly confined to the "kraal." He was run with ewes but did not copulate with them, although he showed sexual interest.

In June, 1928, the ram again began to show listlessness and loss of weight, so that it was decided to take him back to Onderstepoort and graft him again. He was accordingly grafted with four sections —5 by 1.5 x 1 cm.—of testicle from a two-and-a-half years old normal ram on the 12th June, 1928. The operation was done under general anaesthesia using 5 grams of a 10 per cent. solution of chloral hydrate in normal saline intravenously. Complete anaesthesia lasted $1\frac{1}{4}$ hours. The operation was carried out as already described, the grafts being attached to the external surface of the parietal layer of the tunica vaginalis. The wound healed *per primam intentionem.* The grafts could be felt for about six months, but then gradually became absorbed until they could not be palpated.

After this operation the sheep improved somewhat in condition for 6 months. He appeared slightly more active and did not lose weight. At the time of the operation in June, he weighed 121 lb., but had fallen to 119 lb. in December, 1928. The ram was put in contact with two young ewes for 10 months after the operation, but neither proved pregnant. He was not observed to take any sexual interest in them. There was a gradual return to the former state of senility, but his weight remained practically unchanged until August, 1929, when it was decided to do a third operation. At this time the body-weight had dropped to 116 lb.

A third operation was done on the 8th August, 1929, under general anaesthesia; six grams of a 10 per cent. solution of chloral hydrate in normal saline being used intravenously. Anaesthesia lasted 2 hours. This operation was done in a similar manner to the second; four sections of testicle from a two-years-old ram were grafted on to the surface of the parietal layer of the tunica vaginalis. During the operation no trace of the previous grafts was seen.

Both grafts on the right side became necrosed and sloughed away on the 30th August, 1929. The wound on the left side healed *per primam intentionem.* The wound on the right side healed rapidly after the necrosed grafts had been removed.

After the third operation there was slight improvement in the general condition. The weight, however, was not long maintained. There was a gradual loss. The sheep had to be assisted to rise. He usually lay on the right side, and constantly injured the base of the right horn, by striking it against the ground in his efforts to stand.

In November, 1929, symptoms of old age were again just as marked as when the ram was first grafted in June, 1927. It was, therefore, decided to destroy him for post-mortem examination, and histological examination of the grafts.

This improvement in the general condition would appear to substantiate the experience of Falkenheim and Kirsch (1928) who performed Voronoff's operation on a male rat suffering from senile decay. The improvement, however, was entirely temporary and had

disappeared again in six weeks. In the meantime, however, the operated rat fertilised 2 young female rats, although there was complete indifference to female rats showing oestrus prior to the operation. Four and a half months subsequent to the operation the rat died suddenly. A post-mortem examination showed senile atrophy of the testicles and total resorption of the transplanted tissue.

The fate of the grafts examined *in situ* and the histological appearance of grafts three and a half months after implantation have already been described in the previous experiment.

The appended photographs give a good idea of the advance towards senility. The changes in the general appearance of the wool and increased listlessness are very marked.

A detailed description of the post-mortem examination follows:—

P.M. 8728. Sheep. Merino. Ram. 15 years. Condition poor. Weight 105 lb. D.O.B. No. 18342.

Interim	Immediately after death.
Rigor mortis	Absent.
Integumentum	Adherent to the parietal layer of the tunica vaginalis, on the left side two grafts can be seen surrounded by connective tissue, measuring 2·0 × 1·25 × 1·0 cm. On section one of these grafts shows a small cavity filled with a tenacious material; this cavity is about 4 mm. in diameter, and is surrounded by a tough yellowish capsule. The rest of the graft on section is firm and fibrous looking.
Sanguis...	Nothing unusual.
Museuli	Normal.
Subcutis	A little fat present.
Cavum abdominis ...	Nothing unusual.
Diaphragma	Nothing unusual.
Cavum thoracis	Nothing unusual.
Glandulae salivales ...	Nothing unusual.
Organa endocrinata...	Nothing unusual.
Pharyngeal glands ...	Pale and slightly moist.
Bronchial glands... ...	Pale and moist.
Lingua	Mucosa pale, otherwise nothing unusual.
Pharynx	Nothing unusual.
Oesophagus	Mucosa pale, otherwise nothing unusual.
Larynx	Mucosa pale, otherwise nothing unusual.
Aorta	Nothing unusual.
Traehea...	Mucosa pale, otherwise nothing unusual.
Pulmones	Partly inflated, elastic, pleura smooth and pink in colour. On section pinkish in colour; a slight amount of blood-stained froth is present; bronchi contain a small amount of blood-stained foam.
Pericardium	Nothing unusual.
Epicardium	Nothing unusual.
Right ventricle and auriele	Endocardium and valves show nothing unusual.
Left ventricle and auriele	Endocardium and valves show nothing unusual.
Myocardium	Brownish red in colour and of normal consistence.
Hepar	Capsule normal; on section few Stilesia present; colour brownish red; lobulation fairly distinct, lobuli greyish brown, periphery grey. Consistence somewhat firm.
Gall bladder	Contains about 5 c.c. of a greenish liquid bile.
Pancreas	Nothing unusual.
Lien	Measures 14 × 9 × 3 cm. Capsule normal; on section tissue is dark red. Trabeculae and lymph nodes easily seen; consistence normal.
Adrenales	Show a brownish-yellow cortex and a greyish-white medulla; the consistence is normal.

Renes ... ... Show a little perirenal fat; the capsule is easily detached and leaves a smooth brownish red surface; on section the zones are distinct, the cortex is greyish-red in colour, glomeruli not distinct, boundary zone is pinkish-grey in colour. The medulla is the same, and in addition shows marked striation. The consistence is normal. Right kidney as left.
Rumen ... ... Well filled with ingesta, mostly lucerne, mealies, veld hay, and oats; mucosa normal.
Reticulum ... ... Filled with ingesta; mucosa normal.
Omasum... ... ... Some ingesta present; mucosa normal.
Abomasum ... ... Fair amount of ingesta; mucosa slightly pale in colour; a few globidium cysts in the mucosa; one wireworm is present.
Duodenum ... ... Shows pale mucosa.
Intestinum tenue... ... Mucosa is pale, otherwise normal.
Intestinum crasum ... Mucosa is pale, otherwise normal.
Mesenterium ... ... Nothing unusual.
Testicles ... ... Shape normal, but smaller than one would expect in an adult ram. On section it cuts rather firmly, and is brownish in colour. There are a few fibrous adhesions present between the parietal and visceral layer of the tunica vaginalis on the right side.
Brain ... ... Shows nothing unusual.

Pathological Anatomical Diagnosis—

General anaemia as a result of bleeding.
One haemonchus contortus.
Few globidium cysts.
Two grafts adherent to surface of the left tunica vaginalis parietalis.

Etiological Diagnosis.—Killed for post-mortem.

Weights.—Heart .22 kg., liver .5 kg., spleen .19 kg., kidneys .07 kg., lungs .75 kg.

Conclusions.

(1) The general health of a ram showing senility improved considerably after grafting a large section of testicle into the region of the flank. The improvement was maintained for about 9 months. The improvement in general health was not accompanied by increased *libido sexualis;* the ram did not copulate, although he was sexually interested in ewes.

(2) The second operation of grafting into the scrotum caused a temporary increase in bodily vigour, but no increased *libido sexualis.*

(3) The third operation of grafting into the scrotum appeared to have only slight influence in checking advancing senility.

(4) The grafts, whether into the flank or into the scrotum, gradually became obsorbed until no trace of them could be found.

(5) The internal organs on post-mortem examination were in extraordinary good condition for an aged sheep.

(6) There is no doubt that grafting increased the length of life of the sheep.

(7) Grafting to be of practical value must be undertaken before marked symptoms of senility have become evident.

(8) Repeated grafting did not maintain the improvement in health established by the first operation. The improvement was less noticeable after successive graftings.

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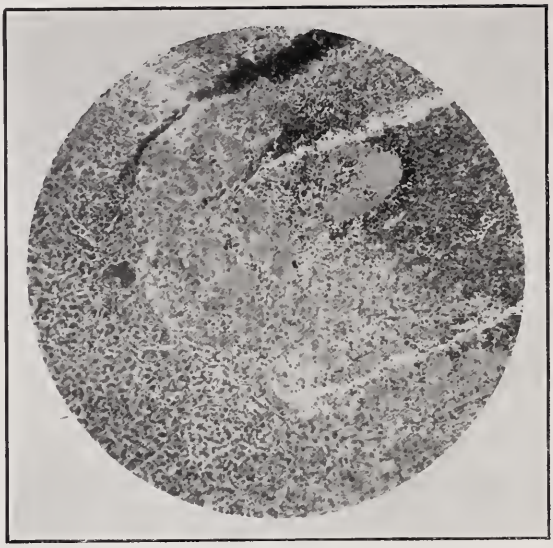

Fig. 1.

Section from centre of a graft showing hyalinisation of the seminiferous tubules. One giant cell can be seen outside the central area. × 105.

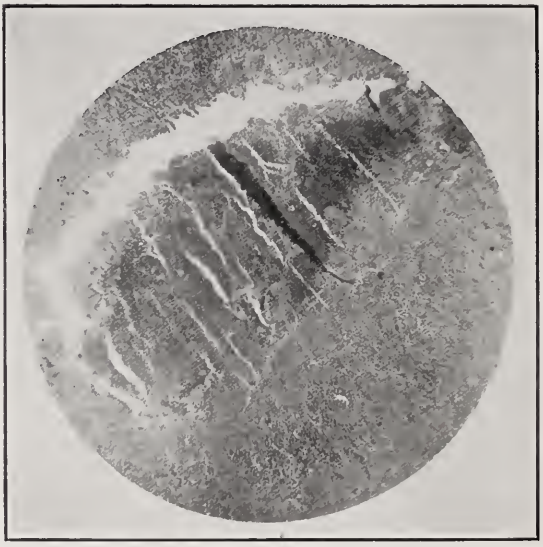

Fig. 2.

Section from the centre of a graft showing the hyalinised remnants of seminiferous tubules. × 30.

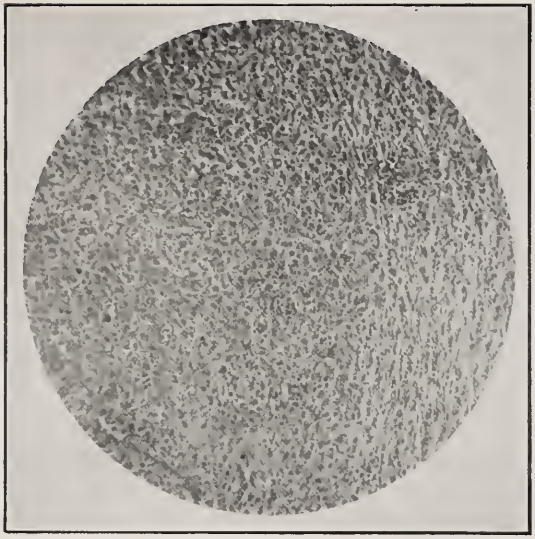

FIG. 3.

Section from the intermediary zone of a graft showing epithelioid cells, round cells and fibroblasts. × 105.

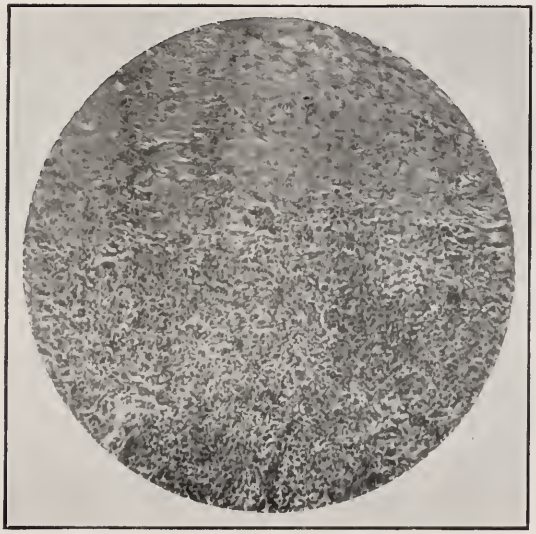

FIG. 4.

Section from the periphery of a graft showing epithelioid cells, round cells and fibroblasts with a surrounding zone of connective tissue. × 105.

FIG. 5.
Section from right testicle of ram 18342 showing aspermatogenesis. × 105.

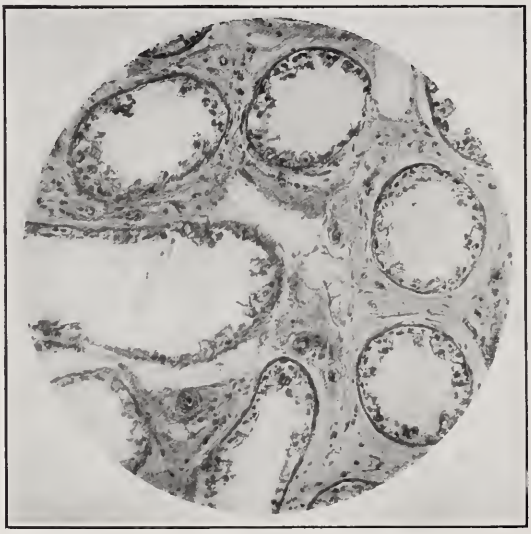

FIG. 6.
Section from left testicle of ram 18342 showing aspermatogenesis. × 105.

FIG. 7.
Rams Nos. T. 402, W. 20, T. 413, W. 30, photographed December 1927, prior to grafting.

FIG. 8.
Control Rams No. W. 9, W. 31, T. 417, February 1928. The similarity of type is apparent, but the rams appear bigger as they are taken nearer the camera.

FIG. 9.
Comparative group of grafted and non-grafted rams. The four grafted rams are on the right of the photograph. September, 1929.

FIG. 10.
Grafted ram, T. 413. October, 1929.

FIG. 11.
Grafted ram W. 20. October, 1929.

FIG. 12.
Grafted ram, T. 402. October, 1929.

FIG. 13.
Grafted ram, W. 30. October, 1929.

FIG. 14.
Non-grafted ram, W. 9. October, 1929.

FIG. 15.
Non-grafted ram, W. 31. October, 1929.

FIG. 16.
Non-grafted ram, W. 49. October, 1929.

FIG. 17.
Non-grafted ram, T. 417. October, 1929.

FIG. 18.
Type of ewe lambs on which the experiment was carried out. December, 1927.

FIG. 19.
Group of ewes after grafting. December, 1927.

FIG. 20.
Group of control, non-grafted ewes. December, 1927.

FIG. 21.
Group A.—Grafted ewes, 18676 to 18724 (even numbers). September, 1929.

FIG. 22.
Group B.—Non-grafted ewes, 18677 to 18725 (odd numbers). September, 1929.

FIG. 23.
Group D.—Control ewes, 3655 and 26 to 49. September, 1929.

FIG. 24.
Group E.—Control ewes, 50 to 74. September, 1929.

FIG. 25.
Group C.—Control ewes, 1 to 25. September, 1929.

FIG. 26.
Lambs of grafted ewes, group A. September, 1929.

FIG. 27.
Lambs of non-grafted ewes, group B. September, 1929.

FIG. 28.
Lambs of group D. September, 1929.

FIG. 29.
Lambs of group E. September, 1929.

FIG. 30.
Lambs of group C. September, 1929.

FIG. 31.
Ram 18342, 13.10.27. Weight 120 lb. Taken 4 weeks after first operation and two days after shearing. Note healed wound in flank.

FIG. 32.

Ram 18342, 14.6.28. Weight 121 lb. Taken the day following second operation. Note the suspensory bandage in position.

FIG. 33.

Ram 18342, 18.4.29. Weight 115 lb. Taken 10 months after second operation Note improved general condition.

FIG. 34.

Ram 18342, 8.8.29. Weight 116 lb. Taken immediately before third operation. Note change in condition.

FIG. 35.

FIG. 36.

Ram 18342, 5.11.29. Weight 107½ lb. Taken 3 months after third operation.
Note advance towards extreme senility.

Section VIII.

Plant Studies and Poisonous Plants.

J. I. Quin	Further Investigations into the Problem of Geeldikkop (*Tribulosis*) in Small Stock.
D. G. Steyn... ...	Pisgoed of Pisgras.
M. Henrici	Mineral and Feeding Stuff Analyses of Grasses of the Eastern Transvaal Highveld.
M. Henrici	The Phosphorus Content of Grasses in the Eastern Transvaal.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

Further Investigations into the Problem of Geeldikkop *(Tribulosis)* in Small Stock.

By J. I. QUIN, D.V.Sc.,
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Previously several reports were published by the author on the results obtained from field experiments in connection with *Tribulus terrestris* poisoning in small stock in South Africa. In these attention was drawn to the insidious manner in which the disease makes its appearance during a certain period of the year provided of course that the necessary concomitant factors were present. What these factors actually are still remains a mystery to be solved. It is, however, more than probable that climatic factors play a rôle perhaps not only in determining the toxicity of the plant but also in influencing the animal's body as is seen from the perculiar localisation of the oedema to the exposed parts of the skin.

It is clear that *Tribulus terrestris* cannot be regarded as a poisonous plant in the ordinary sense of the term. It is widely distributed over the more arid areas of South Africa which incidentally offer grazing to many millions of small stock, both sheep and goats. Furthermore the plant is ravenously eaten and is apparently of a high nutritive value except when geeldikkop makes its appearance and when the results may be disastrous to stock farmers. Not only are large numbers lost but many animals are disfigured and left unthrifty for prolonged periods. Another point to be mentioned is the fact that animals are frequently lost from identical symptoms in areas where no *Tribulus* is found e.g. the Transvaal high veld. The incidence of this condition commonly termed " Dikoor " to differentiate it from the true geeldikkop caused by *Tribulus*, coincides more or less precisely with the latter condition. For this reason it may be advisable to regard geeldikkop as a symptom complex rather than a specific disease, since it, or an almost identical condition, may be encountered on totally different pastures, i.e. there exists the possibility that the same type of poisoning may be caused by totally different plants perhaps in the same way that *Dimorphotheca* (bietou), *Cynodon* (kweek) and *Sorghum* sp. (kaffir corn) all produce hydrocyanic acid poisoning.

Drenching Tests.

While conducting field experiments on Geeldikkip in the Burghersdorp district it was found that the freshly expressed juice from 3 lb. and over of green luxuriant growing *Tribulus* in the fruiting stage when dosed to young sheep caused death within a

few hours. In these cases hardly any premonitory symptoms were noted and the animals usually dying suddenly. On post mortem the only macroscopic change noticed was the intense chocolate brown discolouration of the blood throughout the body.

A quantity of hay was made from the same type of *Tribulus* with which further tests were conducted at Onderstepoort. When offered to stabled sheep the hay was not readily eaten although after a few days fair amounts were taken if nothing else was given. None of these animals, whether stabled or placed in the sun, showed any symptoms even after two weeks. It was then decided to drench the material to sheep through a stomach tube.

Consequently the material was finely ground and mixed up in water. The single doses given in this way were rather small (up to 200 gm.) since the powder took up a large amount of water, necessitating the addition of up to 3 litres of water in order to cause it to flow through the tube. Animals dosed twice or even three times daily failed to show any symptoms even after dosing for a week or longer. It was then decided to dose the watery extracts only, made from larger amounts of hay. The powdered hay was generally soaked overnight in cold water and the watery extracts pressed out through a fruit press. With this fluid it was found that the extracts from 600 gm. and over of the dry *Tribulus* regularly killed young sheep, while adult sheep required 1,000 gm. and more to kill. Again no marked symptoms were noted except that generally about 15-30 minutes before death there was a progressive brown discolouration of all the visible mucous membranes including the conjunctiva. Respiration became faster and shallower, the animal standing quietly in a corner of the stable. There was a fixed staring appearance of the eyes. Just before death the animals showed a swaying unsteady gait, fell down and then passed into a state of coma. Cutaneous reflexes during this period were markedly exaggerated. Breathing now became slow and deep, with frequent expiratory groans, until death supervened. On post mortem again the only striking feature was the marked brown discolouration of the blood. The lungs also appeared a light greyish brown.

When sublethal doses of extract were given it was found that brown discolouration of the mucous membranes accompanied by asphyxial symptoms may in some cases supervene depending on the dose. Recovery in such cases is, however, very rapid e.g. in one case a sheep already in the state of coma was found on its legs and feeding again within one hour.

Dosing of Rabbits.—The same extracts were dosed to numbers of rabbits in amounts up to 200 gm. equivalent of the dry powder. None of these, however, showed any symptoms of brown discolouration of the blood and hence not a single one died from the *Tribulus* extract.

Drenching of Tribulus Extracts in conjunction with Ultra-Violet Radiation of the Animals.

Since the possibility exists that the actinic rays of the sun may in some way influence the incidence of Geeldikkop it was thought advisable to note the effect of ultra-violet rays on the skin of sheep

and other animals both before and after dosing with watery extracts of *Tribulus* as mentioned before. For this purpose the ordinary type of quartz mercury vapour lamp was used. The heads and neck of the sheep were very closely shorn so as to expose the skin as much as possible to the rays. The animals were dosed three times daily at regular intervals with watery extracts up to 400 gm. equivalent of the powdered *Tribulus*. They were then exposed immediately after each dose to ultra-violet radiation for 90 minutes with the top of the head kept at a distance of 20 inches from the lamp. Measures were taken to blindfold all animals so as to prevent injury to the eyes. Generally a light transitory erythema especially of the upper side of the ears, was noticeable after the first radiation. Repeated exposure, however, never itensified the condition nor did it produce burns.

Although a number of sheep were treated in this way for periods up to 14 days, all remained in good health i.e. neither were there any signs of subcutaneous oedema of the head and ears nor was the usual icterus of geeldikkip noticeable. Rabbits similarly dosed and irradiated, only showed erythema and later shedding of the epidermis of the ears, otherwise no effects were noticed.

Drenching Tests with Various Chemicals and Plant Juices.

Bearing in mind the oxidising effects of different chemicals it was decided to treat sheep and rabbits with a few of these and to note the results obtained. Amyl nitrite, sodium nitrite and hydrogen peroxide on intravenous injection resulted in death from apparently the same cause as did the *Tribulus* extracts viz. from an intense brown discolouration of the blood. Even when dosed, sodium nitrite in an amount of 5 gm. caused death within 1-2 hours. It therefore seemed probable that similar blood changes followed after dosing of *Tribulus* extracts.

In order to ascertain whether *Tribulus terrestris* was the only plant which could cause death in such a perculiar manner, various other plant juices were tested, e.g. Young sheep were dosed with pure freshly expressed green lucerne juice in doses of 3 litres each three times daily without showing any ill effect. Similarly the juice from *Cynodon dactylon* (kweek), *Themeda triandra* (rooigras), *Panicum* and *Setaria* species were dosed all without producing any ill effect.

Examination of Blood.

Blood was drawn immediately before and also after death both from cases of *Tribulus* poisoning and also from sheep poisoned with sodium nitrite and H_2O_2.

When the freshly drawn blood was examined spectroscopically i.e. a fairly concentrated haemolysed solution, a definite band in the red was noticed in each case, closely corresponding with that noticed in solutions of methaemoglobin. On standing, this brown blood gradually assumed a red oxyhaemoglobin colour which afterwards turned to the purplish red of reduced haemoglobin. These changes were also verified spectroscopically. Small amounts of ammonium

sulphide when added to this brown solution, whether obtained from a *Tribulus* sheep or from one dead from sodium nitrite, is immediately changed to the bright red of oxyhaemoglobin, and very soon afterwards to the purple red of reduced haemoglobin.

Similar tests were carried out *in vitro*, in which normal blood was drawn from various species of animals and afterwards centrifuged and the red corpuscles washed and then haemolysed. To various dilutions of such Hb. solution small amounts of clear watery extract of *Tribulus* was added, some of which was exposed to sunlight while other tubes were immediately placed in the dark. Spectroscopic examination was carried out at intervals of 5 minutes. As a rule in about 10 minutes the faint dark band in the red could be made out. This, however, became markedly accentuated and was clearly visible within one hour, the whole solution turning a chocolate brown similar to that noted in animals poisoned with *Tribulus*.

In the case of solutions kept in the dark it required 4-5 hours before any changes could be made out and about 12-15 hours before the brown colour was as intense as in the solutions exposed to the sun's rays.

In this way various other plant extracts were also tested and it was found that lucerne as well as all the other grass extracts used produce the brown discolouration of the Hb. solutions in *vitro* although the time required was definitely longer than in the case of *Tribulus* extracts.

Summary.

Although various experiments were carried out in an attempt to produce geeldikkop cases by drenching fresh, green *Tribulus* juice, or the watery extracts from dried *Tribulus* no cases of dikkop could be produced. Sheep could, however, be easily killed apparently from a condition of methaemoglobinaemia in which the oxygen-carrying capacity of the blood was markedly interfered with, and resulting in acute asphyxia. Whether this condition has any relation to the oedema and icterus noted in Dikkop is at present not known. In no case was haemolysis noted, the plasma remaining absolutely clear, i.e. the change seems to be solely intraglobular and does not tend to cause disintegration of the red cells.

Further experiments are being conducted especially in connection with the role played by the liver and digestive tract, in the production of this peculiar condition.

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Pisgoed or Pisgras.

By D. G. STEYN, B.Sc., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

As early as 1772 Sparrman mentioned " pisgras " causing a stoppage of urine frequently with fatal results. The disease occurred on the northern boundary of the Mossel Bay District and nobody could with any degree of certainty point out the responsible plant. He suspected it to be a Euphorbia species. Both in the urine and urethra of the affected animals little lumps of a cheesy material had been observed and the only chance of saving the animal had been by driving it about in order to expel the coagulated matter.

Watt in " The History of Pharmacology in South Africa " states that Thunberg held that *Euphorbia genistoides* contains a milk which causes no trouble in the stomach and bowels of cattle but corrodes the bladder and obstructs the urinary passages of the ox and horse causing strangury.

In the past " pisgoed or pisgras " has always been considered to be *Euphorbia genistoides*. Recent investigations, however, have revealed that the term " pisgoed " is used by farmers to describe practically all the inflammatory processes of the external and internal genital organs of the ram, wethers, he-goats, castrated goats and oxen. Many farmers hold a plant (named " pisgoed " by them) responsible for the disease, but a peculiar fact is that when they are questioned as to the nature of the plant, nobody can describe it or point it out.

As the Director of Veterinary Services was anxious that the aetiology of the disease commonly called " pisgoed " should be cleared up, it was decided to investigate a heavy outbreak of " pisgoed " on the farm Doffenspruit, Maquassi District, and it is proposed to discuss this investigation and the subsequent experiments which were conducted with six " pisgoed " cases forwarded to Onderstepoort by Mr. Hellmuth, Doornpan, P.O. Bultfontein, Orange Free State.

Morbidity.—Of four hundred merino wethers, over three hundred were affected and ten had already died.

Mortality.—The mortality depends on the severity of the disease. Moist and hot weather render the conditions for the spreading of the disease very favourable and it was actually during the hottest and most wet months of the year that most outbreaks were reported.

Symptoms.—The disease sets in with a redenning and swelling of that part of the preputium which is in immediate contact with the wool. The inflammation then spreads and frequently finds its way into the sheath and causes great pain to the animal. Owing to the *orificium preputii* being constricted by the swelling, the urine accumulates in the sheath, and the products of decomposition of the retained urine markedly aggravate the condition by causing severe irritation on the already inflamed mucosa of the sheath. If such cases are not treated in time they end fatally almost without exception.

Within a day or two small vesicles filled with a clear watery liquid appear on the reddened and swollen skin and visible preputial mucous membrane. These vesicles, however, become pustules within a very short time. These pustules then burst and yellow ulcers are formed which, in the course of time, are covered by yellowish brown crusts underneath which yellow pus accumulates. On removing these crusts there remains a bleeding purulent surface.

In cases where the inflammation has spread into the sheath large amounts of a very bad smelling yellowish pus can be expressed from the swollen and distended sheath.

The wool immediately surrounding the preputium is moist with urine and the products of the inflammation, and it emits a most offensive smell.

The general health of the animals is affected only in the worst cases. The cause of death is a toxaemia.

Post-mortem lesions.—No animals were post-mortemed on the farm Doffenspruit, but autopsies were conducted on three of six "pisgoed" wethers (Nos. 24828 to 24833) sent to Onderstepoort by Mr. Hellmuth. These animals were carefully examined and found to be suffering from a disease identical with that found on the farm of Mr. Kirsten, Doffenspruit, Transvaal.

The post-mortems revealed that the animals were badly affected with *Haemonchus contortus*, *Oesophagostomum columbianum*, *Trichostrongylus*, and *Stilesia hepatica*. The lesions on the genital organs were identical with those described under "Symptoms." No calculi or lesions other than those described above could be found in the urogenital tract.

EXPERIMENTS CONDUCTED AT ONDERSTEPOORT.

The following experiments were conducted with the sheep forwarded by Mr. Hellmuth:—

I. *To ascertain whether "pisgoed" is infectious*: Pus collected from wether No. 24830 was rubbed on to the *orificium preputii* and preputium of two long-woolled wethers (Nos. 23781 and 24404). Within 24 hours after the application of the pus both animals showed reddened and swollen *preputia*, and within another 24 hours small vesicles appeared at the site of inflammation. The affection took the same course as that described in the natural cases.

Case No. 23781 recovered without treatment within eight days after the pus had been applied to the *preputium*, but case No. 24404 had to be treated as described under "Treatment."

II. *To ascertain whether it was possible to produce the disease under stable conditions*: The wool of wethers Nos. 19751, 20212, 20753 and 20038 immediately surrounding the *preputium* was tied in such a way as to allow it to be moistened by urine and to remain in constant contact with the *preputium*. Within 48 hours all the animals showed pronounced reddening and swelling of the *preputia* and the further development of the disease was identical with the natural cases. These four cases were very bad and as the inflammation was extending into the sheath, it was decided to treat them in time so as not to cause the animals unnecessary pain.

Two wethers Nos. 19074 and 19518, were treated as above, and in addition pus collected from the natural case No. 24830 was applied to the *preputia*. Both these animals showed lesions as described above and had to be treated as there was no sign of improvement.

III. *To ascertain whether any definite organism or organisms are responsible for this disease*: Dr. Robinson of the Bacteriological Department made blood-serum-agar cultures from three of the above cases and found cocci and streptococci. The cultures were applied to the *preputia* of wethers Nos. 20148, 22115 and 21165 with completely negative results.

Aetiology.—Examination of the pastures on the farm Doffenspruit on which the animals were running revealed no plants that one could possibly suspect to be the causative factor. It was definitely proved (*a*) that the disease could easily be transmitted from affected to healthy animals, and (*b*) that the disease could be produced without any difficulty, simply by tying the wool round the *preputium* so as to allow the products of decomposition of the urine to act on and irritate the skin of the *preputium*. It was invariably found that the inflammation set in at those points where the skin was in immediate contact with the moistened and bad smelling wool. It was found that the disease, provided it had not progressed too far, could easily be cured by removing the causative factor, namely the moist and offensive smelling wool surrounding the *preputium* and by applying a disinfecting ointment.

It was furthermore proved that the causative organisms had attained such a degree of virulency that they, when brought on to the preputia of normal wethers, were able to cause the disease without the co-operation of the predisposing factor, namely, the irritation caused by the products of decomposition of the urine.

The term "pisgoed" as used by the farmers, no doubt includes quite a number of diseases, as for example, urinary calculi found in the urogenital tract, and it seems as if this term was originally intended to describe this condition only, but at present it is used to

describe practically all diseases of the urogenital passages. I would like to point out that this article treats with " pisgoed " which is caused by the continual moistening of the wool immediately surrounding the *preputium* resulting in constant irritation of the skin by the products of decomposition of the urine.

It would be greatly appreciated if our colleagues in the field should supply us with all the available information as regards this disease as it would be of great interest to ascertain experimentally, whether there is any truth in the contention that plants (for example, *Euphorbia genistoides*) are responsible for the formation of urinary calculi, which in turn cause a urethritis. It is possible that the disease may set in with a few cases in a flock, and that the bacteria may in the course of time become so virulent as to attack short-woolled wethers without the assistance of the predisposing factor. All the cases which have been brought to our notice in the past occurred in long-woolled wethers.

Treatment.—The treatment of the type of " pisgoed " described above is obvious. The most essential part of the treatment is to clip the wool surrounding the *preputium* as short as possible. The scabs and pus must be removed with cotton wool, and an ointment containing a disinfectant must be applied. Very satisfactory results were obtained at Onderstepoort with an ointment prepared from vaseline or lard (9 parts) and carbolic acid or Little's Dip (1 part). It is essential that an ointment should be used as it is soothing and also adheres to the inflamed surfaces for a longer time than preparations in liquid form will. When the inflammation has extended into the sheath, the pus should be expressed and it would be advisable to inject into the sheath an oil (olive oil or raw linseed oil) mixed with a disinfectant. The above treatment must be repeated as often as is considered necessary.

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Mineral and Feeding Stuff Analyses of Grasses of the Eastern Transvaal Highveld.

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The following analyses were done to characterize the veld grasses of the district of Ermelo in the Eastern Transvaal as to their content of lime, magnesia, potassium, sodium, chlorine and phosphorus as well as to their content of crude protein, fibre, ash, fat and carbohydrate. It was not intended to investigate the eventual migration of the minerals during the season, thus all the values are calculated on stove dry matter. Samples of the green and brown leaves of representative grasses were collected either at intervals of three or four weeks or only a few times a year, the time available for the work being limited. The samples, generally about 2 lb. dry matter, were carefully dried at 104° C., the material kept in a desiccator and weighed out for the particular analysis.

Most analyses were done on *Themeda triandra*, *Monocymbium cerisaeforme*, *Digitaria tricholaenoides*, Stapf., and *Hyparrhenia Buchanani*, Stapf., and some fewer on mixed veld grass, on *Cynodon Dactylon*, *Panicum laevifolium*, and on an Eragrostis. Samples of Themeda were taken from different soils. Further samples were collected from unfertilized mealies to compare their mineral content with that of veld grass on the same soil. Themeda, of course, was selected as the dominating grass in this area; some of the other grasses proved to be true enough samples of the area, but revealed in their analysis that cattle cannot thrive solely on them, at least at certain times of the year. As a matter of fact *Harpechloa capensis*, Kunth, was chosen because of a remark of Bews (1926, p. 146), who thought it might prove to be a good grass.

The type of veld and the meteorological conditions of the farm Nooitgedacht 10, at Ermelo in the Eastern Transvaal, have been described in a previous paper (Henrici 1930) to which the reader is here referred.

The rainfall, however, may be fully mentioned.

1926.	c.c.	1927.	c.c.	1928.	c.c.
July	0·21	January	11·18	January	10·61
August	0·0	February	7·95	February	11·65
September	10·4	March	7·7	March	18·8
October	2·7	April	3·32	April	2·26
November	8·31	May	2·31	May	2·94
December	11·12	June	0·0	June	0·0
		July	6·34	July	0·0
		August	0·0	August	0·42
		September	2·6	September	4·81
		October	16·47	October	1·35
		November	4·68		
		December	·62		

From the above table it can be seen that in the season 1927-28 there were two periods in Ermelo, in which heavy rains had fallen, in October and in March, 1928. In the season 1926-27 the rain was more evenly distributed.

THE METHODS.

For the analytical work different well-known methods were used, sometimes with slight modifications which proved necessary. For the feeding-stuff analyses the official and tentative methods of analysis of the Association of Official Agricultural Chemists (1925) were used. The calcium and magnesium were determined according to McCrudden (1910). Some of the samples contained more iron and manganese than is allowed for with McCrudden's method, thus these two minerals had to be removed with ammonium acetate (see Abderhalden I/3, p. 728 ff.). The determination of potassium and sodium was done in an aqueous extract, as described by Kostytchew (1926, p. 287), all the alkalis in the leaves are present in water soluble form. Some special analyses were done to test this point. For the water extract, 10-20 gms. of material were extracted with 500 c.c. of water for several hours on the waterbath, and filtered. The filtrate, with the addition of some concentrated hydrochloric acid was then evaporated down to dryness and ignited in a double basin on an alcohol burner, great care being taken not to exceed 700° C. The ash was moistened from time to time with water or hydrochloric acid in order to make it whiter, as the first ashing rarely produced a perfectly white ash. It was taken up with hot water, filtered, the solution evaporated down, and some drops of dilute ammonia and 1.0 c.c. of 5 per cent. ammonium carbonate added to precipitate calcium. The warm solution was filtered, the filtrate again evaporated almost to dryness, 1-3 drops of hydrochloric acid added and ammonia for neutralizing, and 1 c.c. of 3 per cent. ammonium oxalate added to remove the rest of the calcium. The whole suspension was evaporated to dryness and gently ignited. The residue was taken up with water and filtered. Evaporating and igniting had to be repeated several times to cause all the insoluble material to come down, a rather tedious task. Finally the chlorides were weighed. The chlorides consisted chiefly of KCl; NaCl was present in only small quantities most of the time. As it was of special interest to veterinarians, potassium and sodium were separated by methods used in the Onderstepoort laboratory by Dr. H. H. Green.

The chlorides were dissolved in water and a very small aliquot taken for the determination of potassium, which was then determined by the following method. The aliquot is acidified with acetic acid and 1 c.c. surplus acetic acid added. Then 10 c.c. sodium cobaltinitrite (see below) are added. The yellow precipitate with the matrix is then evaporated down until the purple syrup sets stiff on cooling. The cold mass, consisting of the precipitate of $K_2NaCo(NO_3)6{\cdot}H_2O$ embedded in a dark purple syrup, is stirred up and allowed to soak with 50 c.c. 10 per cent. acetic acid, until the excess sodium cobaltinitrite is completely dissolved. The precipitate is then filtered by suction through asbestos. An excess (30-50 c.c.) of N/5 potassium permanganate is heated to boiling in about 300 c.c. of water, plus 20 c.c. of 25 per cent. sulphuric acid. Precipitate and asbestos are

then dropped into the boiling solution, and gently boiled for about five minutes, i.e. until the yellow precipitate is completely dissolved. Manganese hydroxide separates out. Standard N/5 oxalic acid is then run in from a burette until the solution is colourless. As the exact end point is difficult to hit, a few c.c. excess of oxalic acid are usually added, the excess being determined by back titration with N/5 potassium permanganate. The difference between the amounts of permanganate and oxalic acid gives the amount of permanganate used by the potassium, 1 c.c. of N/5 permanganate corresponding to 0.001714 gm. K_2O.

The cobalti-nitrite reagent for potassium is made up as follows:

113 gm. of Cobalt acetate are dissolved in 400 c.c. of water and 100 c.c. glacial acetic acid are added; 220 gm. of sodium nitrite are dissolved separately in 400 c.c. of water and the two solutions are mixed while stirring. NO_2 is removed completely by evacuation or by blowing air through the solution. After 24 hours any sediment is filtered off and the volume diluted to 1 litre. The solution is dark plum colour and must be kept in the dark.

The sodium was determined by evaporating down the remainder of the solution of the total chlorides to a bulk of 2 or 3 c.c. in platinum or silica basins, and applying the method of Kramer and Tisdall (1921).

The direct determination of K and Na was not always quite satisfactory in spite of every precaution being taken. Only results were accepted in which the sum of the chlorides of the seperate determination added up to the amount of chlorides originally found. The amount of chlorides found in 10 gm. of green leaves varied from 0.2-0.4 gm. and the sum of the chlorides separately determined should not differ by more than 0.010 gm. The limit is rather large, but with the present method of analysis better results could not be anticipated. The result is sufficiently clear to show what quantities of these two minerals are present in the plant.

The phosphorus was determined by the method described before (Henrici, 1930). The figures are given here merely to complete the table. The yearly variation was discussed in a previous paper.

For the chlorine Tschopp's micro-method was used. As this method has not been used very often for plant work, some more information might be given. For the grasses which were rather poor in chlorine 0.5 gm. is combusted with 10 c.c. of 0.02/N Silver nitrate, 5 c.c. conc. nitric acid and 2 c.c. of Merck's G.R. 30 per cent. Perhydrol. More acid or perhydrol were very seldom used.

Tschopp's prescription to add 1 c.c. fuming nitric acid to the titration had to be modified, as no fuming nitric acid was obtainable in South Africa. Merck's conc. HNO_3, G.R. was used.

The whole combustion, in 100 c.c. Kjeldahl flasks, takes 40-80 minutes on a hot plate at 300° C. The analysis is most satisfactory. The yellowish solution is filtered and made up to 50 c.c., 20 c.c. of which are titrated with 0.02/N KI in a microburette, taking from 2-4 c.c. KI. For other plants, e.g. Karroo bushes which contain much more chlorides, less material, more silver nitrate and a smaller aliquot of the filtrate have to be taken.

A criterion of the amount of acid to be used is that in the final titration, the first drop of KI produces an immediate blue colour with the starch, which disappears on shaking, until the reaction is complete.

RESULTS.

The results are given in Tables 1, 2 and 3. Table 1 contains the total and mineral analyses of the two grasses which were regularly analysed during a whole year, Table 2 is a compilation of all data on the other grasses, while Table 3 contains some comparative data on the alkalies of unfertilized mealies.

TABLE 4.

Soils of Nooitgedacht 10, *Ermelo District, Transvaal.*

	Grey Soil. Camp B.	Dark Brown Soil. Camp B.	Black Soil. Camp H.	Grey Soil. Camp Q.	Red Sandy Soil. Camp O.
Moisture	0·46	0·91	6·42	0·74	1·86
Loss on ignition	2·67	4·77	5·79	2·04	5·15
Insoluble matter	91·30	87·00	73·18	94·37	75·80
Iron and aluminium oxide	1·84 2·90	1·8 4·63	12·84	2·52	15·52
CaO	0·05	0·06	0·70	0·06	0·07
MgO	0·08	0·08	0·82	0·07	0·14
K_2O	0·15	0·27	0·12	0·03	0·12
P_2O_5	0·03	0·03	0·03	0·03	0·07
Soluble silica	0·23	0·22	0·31	0·09	1·08
Nitrogen	0·078	0·193	0·084	0·056	0·090
Available K_2O	0·012	0·007	0·0035	0·0128	0·0131
Available P_2O_5	0·001	0·002	0·0012	0·0014	0·0007
Available CaO	0·010	0·016	0·326	0·023	0·046
Available MgO	0·009	0·010	0·152	0·014	0·027
PH	5·2	5·1	—	—	—

The " total " analyses of Table 1 do not offer anything striking for South African grasses. The figures approximate those found by Marchand and Smith (1909), and Vipond (1914). Compared with data from the Bechuanaland farm, Armoedsvlakte, however it is obvious that throughout the year both grasses are much poorer in crude protein and ether extract, and rather richer in fibre and N-free extractives (Theiler, Green and du Toit, 1924, p. 29, and Henrici, 1928). This means that the dominating grasses of the Highveld in Ermelo have never the same feeding value as the grasses of the dry Bechuanaland. This is rather an unexpected result considering the much higher rainfall of the Eastern Transvaal. The results obtained in the winter months especially compare unfavourably with the Bechuanaland data.

Data on the mineral content of South African grasses are not sufficient for ready comparison. If the data obtained, however, are compared with those for Great Britain (Godden, 1926), one can only wonder that deficiency diseases amongst animals and plants are not even more common in this area than is the case. In Table 4 it can be seen that for calcium and magnesium in all the soils investigated the magnesium content is higher than the calcium content; the available magnesium is about $\frac{2}{3}$ of the available calcium, except for the

black soil of H camp. It is generally accepted that the ratio Ca: Mg = 3:1 is the best for plant life, although a number of exceptions are known. (Czapek, 1920, p. 497.) As can be seen from the table the soils are acid. It is not likely that such soil conditions are very favourable for plant life.

The relation of calcium to magnesium in the leaves is similar to that in the soil. In green leaves magnesium may be present in the same amount, or even slightly more, or at least $\frac{2}{3}$ of the calcium. Only the Themeda in the black soil has a better relation in the older green leaves. There is no doubt that the calcium content of nearly all the Ermelo grasses is very low. The pasture therefore has to be included among those poor in calcium and phosphorus. *Hyparrhenia Buchanani* is the only grass which gives values over 0.5 per cent. for the calcium content of its green leaves.

The seasonal variation of the calcium does not seem to be the same for all plants. Cruickshank (1926) and Woodman (1928, 1929) found a maximum in pasture grasses in Great Britain in the summer. Czapek (1920), in a resumè of the literature states that a continuous increase of the calcium takes place with the advancing season. In the literature the accumulation of calcium in old brown leaves is generally accepted. In some of the investigated grasses a late spring maximum of calcium is observed (*Themeda triandra* in O and H camps, and *Cynodon Dactylon*); in other grasses it is decidedly not seen (*Themeda*, B camp, *Hyparrhenia Buchanani* and *Monocymbium*, B camp). The accumulation of calcium in autumn and winter is distinct in nearly all the grasses, being especially pronounced in *Digitaria tricholaenoides* and *Hyparrhenia Buchanani*. During the winter some calcium may be leached out of the brown leaves. The increase however of calcium is nowhere as much as in Europe, neither does the ash content of the leaves increase in the same way. Digitaria is the only grass which shows a considerable increase in calcium, but no increase in ash content runs parallel.

The seasonal variation of magnesium is different. An increase with the advancing season with a final rapid decrease in autumn (Czapek, 1920, p. 441) is not observed in the investigated grasses. At any time of the season older green leaves contain less magnesium than young green leaves. Brown leaves in winter contain a minimum amount of magnesium. Magnesium is withdrawn in autumn from green leaves (see especially Themeda in H and B camp, Monocymbium B camp, Eragrostis and Digitaria). This different migration has the result that the ratio of calcium to magnesium is especially unfavourable in midsummer.

On the whole the calcium figures are decidedly lower than those of Bechuanaland grasses (see Theiler, Green and du Toit, 1924, p. 29.) The few data available for Rhodesian grasses (Husband, 1929) reveal the fact that for the same time of the year, they are about the same (p. 1007 and 1009). In different years the calcium content of grasses may vary, as has already been pointed out by Woodman (1929.)

The figures for magnesia are on the whole rather higher than the corresponding figures of Bechuanaland (unpublished record). For animal nutrition magnesium will always be present in sufficient quantity (Orr, 1929, p. 26.)

With regard to the alkalies, it appears that potassium is the one mineral which is not deficient in these grasses. The figures for potassium compare quite favourably with those of Great Britain (Godden, 1926.) In the green leaves of Themeda the potassium content does not vary much during the season, decreasing, of course, in brown leaves. There seems to be a maximum in January, although not very pronounced. Digitaria shows two decided maxima, the one in November, the other in March, both at the time of heavy rains when this grass was best developed. As neither the calcium nor the total ash content increase with the advancing season the decrease of potassium calculated on ash, as observed in Europe, is scarcely noticeable in these grasses. A considerable drop occurs in autumn. Brown leaves contain hardly any potassium, especially during the winter months. It is not considered that this is due only to remigration of the K, but surely in winter leaching of the leaves has a lot to do with it; the potassium in the plant is all water soluble and the dying or dead leaf losing the semi-permeability of its membranes does not protect the salt from washing out by dew, rain or frost.

Contrary to the potassium content most grasses of the Highveld contain but little sodium.

The sodium percentage of the grasses is not constant and varies to a considerable degree in samples from different years. Czapek (1920, p. 436) also mentions the great variations of sodium content. The available time was too short to make a detailed study of this question, but some observations may be discussed. It is obvious that on one and the same soil different grasses take up different amounts of Na_2O. (Compare especially *Panicum laevifolium* with *Eragrostis Abyssinica*.) These differences are enormous, and yet no reasons for them can be given. Secondly, there are grasses which seem always to have a low Na_2O content (*Hyparrhenia Buchanani* and *Themeda* in Q and H camps.) In Table 1 it can be seen, that *Themeda*, B camp, and *Digitaria tricholaenoides* contain considerable amounts of Na_2O in young leaves, but that old, especially leached, brown leaves are very poor. With the exceptions already mentioned and that of *Monocymbium*, the sodium oxide content of these grasses never exceeds 0.05 per cent. and is generally much lower. It is very peculiar that all 1926 samples are very low in sodium—the samples were collected after heavy rains and were fresh and green at the end of April. It may be that decreased transpiration lowers the content of Na. This would also explain why spring values at the time of the highest transpiration power of grasses, are rather high. It has to be remembered that sodium is not essential for the life of most higher plants and has no special function in the life of the plant. Sodium is probably absorbed without any special selection and if much water has to be taken up, sodium will be quite passively transported into the leaves.

Compared with data from Rhodesia (Husband, 1929, p. 1009) or with data from Great Britain, the sodium content is indeed very low and the ratio of potassium to sodium strikingly high. It is not impossible that the low sodium content has some effect on the grazing animal.

It may be interesting to mention here the sodium and potassium content of maize grown without manuring on the same soil as these grasses (Table 3). As can be seen from the table the sodium content is much higher than that of the grasses, and reaches the value of European pastures. The potassium content is higher as well, but the difference between the figures for maize and for veld grasses is by no means as striking as that for sodium. The maize plants were healthy looking, but the crop of seed was rather poor, probably due to lack of phosphorus in the soil.

The phosphorus content of Ermelo grasses has been discussed at full length in a previous paper (Henrici, 1930), thus it need be dealt with only shortly here. There does not seem to be any relation between phosphorus content and calcium content. It is true that in young green leaves calcium and phosphorus content are both high, but old leaves are high in calcium and low in phosphorus so that this similarity does not hold good for long.

The chlorine content of the investigated grasses is rather interesting. There is a decided maximum in the young spring leaves; afterwards the chlorine content decreases continually, although after heavy rains late in the season the leaves may show an increase, but the percentage of chlorine in March is never as high as in spring time. Brown leaves are poor in chlorine; especially during the winter and early spring they are practically emptied of all their chlorine. Broadly speaking, the chlorine of the investigated grasses behaves very similarly to the phosphorus, their maxima and minima falling together. One difference exists, in that phosphorus is never withdrawn or leached out from the leaves to the same extent as chlorine.

There is no doubt that there are plants which on the same soil contain very different amounts of chlorine. The values for *Monocymbium* in B camp never reach those for *Themeda*. On the other hand, it is not a characteristic of a good grass, to contain higher amounts of chlorine. *Harpechloa capensis* which proved to be an impalatable grass except in the youngest state, is not poor in chlorine (Table 2.) The only high value late in the season is reached by *Panicum laerifolium*, curiously enough by the grass with the highest sodium content. According to Czapek (1920, p. 451) a high chlorine content need not necessarily be accompanied by a high sodium content, an observation which is fully confirmed by this investigation.

The significance of chlorine for the plant is not yet clear. The newer view that chlorine is not necessary for plant life seems to have a number of exceptions (Czapek, 1920, p. 518 ff.). The observation that with a deficiency of chlorine no seeds are formed in *Fagopyrum*, *Avena* and *Hordeum* deserves full attention, even though it proves to be only due to an indirect influence of the chlorine. When it is remembered how few seeds are formed in the grassveld of Ermelo (Henrici, 1930), I), it does not seem impossible that chlorine has something to do with it. The seed formation of some grasses can be forced by manuring with bonemeal, but peculiarly enough the experiment was a failure in the Highveld of Ermelo, and in Bechuanaland it was by no means entirely successful.

It has already been suggested on a previous occasion that another factor, as yet unknown, influences the seed formation. Further investigation has to show whether or not this factor be deficiency of chlorine. In the Karroo, under much more adverse conditions with regard to rain, plenty of seeds are formed by bushes which contain at least five times as much chlorine, but also by grasses which contain at the time of seed formation about double the amount. Of course the phosphorus content of these bushes and grasses is considerably higher than that of the Ermelo grasses, thus the question has to be studied very carefully.

Not many data are available for comparing the chlorine content of the investigated grasses. The spring figures reach the values of hill pasture grass (Godden, 1926), the autumn figures being distressingly lower; they are however higher that the corresponding figures for Rhodesia (Husband, 1929). The principal difference from European figures lies in the seasonal fluctuations. Cruickshank (1926) states definitely that chlorides do not show a seasonal fluctuation, and Vandevelde (cited after Czapek, 1920, p. 451) also did not find a regularity in the fluctuations.

CONCLUSIONS.

The conclusion must therefore be drawn that the mineral analysis of grasses in the Eastern Transvaal highveld reveals the fact that from the pastoral point of view, this country is rather a poor one.

With the sole exception of potassium, the minerals important for the sustenance of animal life are deficient in the dominating grasses for the greater part of the year.

Little is known as to the specific function of the various minerals in the life of the plant itself. Sodium has been proved to be without importance; the rôle of chlorine is still a debatable point. All that can be said from this aspect is, therefore, that the ratio of magnesium to calcium seems unfavourable and that phosphorus is decidely deficient.

Much worse things might justifiably be said by the farmer who has to rear sheep or cattle on this pasture, and for whom the mineral value of his pasture is of primary importance. The phosphorus problem is vital, the sodium deficiency may have to be taken into consideration, as well as the fluctuations of the chlorine, and the low calcium content is a disadvantage.

For a short time in spring, in the month of October, the highveld pasture may be said to be good; in winter it is very bad, a fact which of course has long been realized by the practical farmer.

RESUME.

Feeding stuff analyses on grasses of Ermelo in the Eastern Transvaal reveal the fact that throughout the year these grasses are poorer in crude protein and ether extract than grasses of Bechuanaland. In spite of the higher rainfall in the Eastern Transvaal the fibre content of the investigated grasses is high.

The mineral analyses show that the investigated grasses are rather poor in calcium and that the ratio of Ca:Mg is narrow. The relation of K:Na however is exceptionally wide, as the grasses reach European figures in their potassium content, but are mostly very poor in sodium. The sodium content of the grasses is not constant and does not show a definite fluctuation during the season. Phosphorus and chlorine show a definite seasonal fluctuation, with maximum values in young spring leaves, then a steady decrease with the season.

Drought depresses the phosphorus content of the pasture (Orr, 1929, p. 41; Henrici, 1928), but drought in the Highveld is never as severe as in many other parts of South Africa, and the summer minimum of phosphorus is not as pronounced as in Bechuanaland.

After heavy rains late in the season the chlorine and phosphorus content may rise again without however reaching the spring values. The lowest values of chlorine are found in old leached brown leaves.

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TABLE 1.—TOTAL ANALYSIS ON ERMELO GRASSES IN PERCENTAGE OF DRY MATTER.

No.	Organ.	Date.	Ash.	Crude Protein.	Ether Extract.	Crude Fibre.	Carbo-hydrates.	CaO.	MgO.	Na_2O.	K_2O.	P_2O_5.	Cl.
	(*a*) *Themeda triandra* of B Camp.												
786	Green leaves	19.1.27	6·81	6·88	3·40	29·82	53·08	0·24	0·24	0·009	1·35	0·196	0·561
1034	Green leaves	21.2.27	7·36	7·06	3·06	30·53	51·99	0·33	0·28	—	—	0·228	0·428
1095	Green leaves	15.3.27	7·13	7·85	2·96	29·73	52·34	0·33	0·22	—	1·24	0·224	0·400
1211	Green leaves	22.4.27	7·54	7·33	2·79	30·25	52·10	—	—	0·009	1·06	0·244	0·196
1212	Old leaves	22.4.27	11·20	2·68	2·53	29·39	54·20	—	—	0·007	1·06	0·084	—
1367	Green leaves	18.5.27	6·66	5·59	2·56	28·98	56·22	0·40	0·29	0·010	1·17	0·228	0·419
1368	Old leaves	18.5.27	9·94	2·74	2·26	30·55	54·45	0·42	0·31	—	—	0·072	0·170
1447	Old leaves	16.6.27	11·62	2·21	2·08	32·48	51·62	0·32	0·22	—	—	0·057	0·202
1514	Old leaves	18.7.27	10·42	2·40	1·54	28·55	57·09	0·43	0·24	—	—	0·054	0·159
1630	Old leaves	15.8.27	10·72	2·32	1·51	30·35	55·10	0·33	0·25	—	—	0·061	0·244
1827	Green leaves	18.10.27	8·78	9·82	2·92	30·06	48·48	0·45	0·33	0·052	1·29	0·330	0·533
1828	Old leaves	18.10.27	11·00	2·49	1·82	31·12	53·57	0·32	0·18	—	—	0·059	0·279
1854	Green leaves	15.11.27	9·04	7·42	2·66	29·82	51·06	0·41	0·15	0·13	1·24	0·238	0·533
1855	Old leaves	15.11.27	11·30	2·47	1·62	31·50	53·11	0·33	0·22	0·036	0·13	0·052	0·008
1872	Green leaves	16.12.27	8·46	8·03	2·47	30·35	50·69	0·39	0·30	—	—	0·316	0·533
1873	Old leaves	16.12.27	16·06	2·76	1·71	28·64	50·83	—	—	—	—	0·068	—
1890	Green leaves	16.1.28	9·01	7·11	2·28	31·37	50·23	0·34	0·29	—	—	0·233	0·192
1891	Brown leaves	16.1.28	—	—	—	—	—	—	—	—	—	0·068	0·088
1942	Green leaves	16.2.28	8·92	7·03	1·97	30·23	51·85	0·40	0·31	—	—	0·240	0·294
1943	Old leaves	16.2.28	10·28	2·65	1·94	29·81	55·32	0·45	0·25	—	—	0·061	—
1998	Green leaves	16.3.28	8·06	7·51	2·14	31·26	51·03	0·27	0·30	0·005	1·38	0·256	0·369
1999	Old leaves	16.3.28	8·85	2·76	2·05	31·52	54·82	0·38	0·21	—	—	0·092	0·277
	(*b*) *Digitaria tricholaenoides* of X Camp.												
1695	Old leaves	1.9.27	7·53	3·86	2·50	31·68	54·43	1·03	0·57	0·005	0·24	0·107	0·016
1757	Green leaves	22.9.27	7·85	13·42	2·85	25·62	50·26	—	—	—	—	0·524	—
1833	Green leaves	24.10.27	—	9·56	—	—	—	—	—	0·11	1·47	0·360	0·657
1834	Brown leaves	24.10.27	—	2·97	—	—	—	—	—	—	—	0·100	—
1860	Green leaves	21.11.27	8·02	9·52	2·51	30·52	49·43	0·37	0·56	0·043	1·70	0·304	0·516
1878	Green leaves	20.12.27	8·06	8·82	2·82	31·97	48·33	0·37	0·51	0·048	1·60	0·254	0·675
1895	Green leaves	23.1.28	9·16	6·38	2·49	31·84	50·13	0·40	0·39	—	—	0·236	0·202
1947	Green leaves	20.2.28	7·38	5·69	2·57	31·84	52·52	0·42	0·46	0·07	1·16	0·212	0·440
1948	Brown leaves	20.2.28	—	3·75	—	—	—	—	—	—	—	0·109	0·068
2003	Green leaves	20.3.28	8·09	4·90	2·49	30·82	53·70	0·36	0·35	0·030	1·70	0·214	0·391
2004	Brown leaves	20.3.28	—	2·45	—	—	—	—	—	—	—	0·090	—
2020	Green leaves	20.4.28	7·47	3·78	2·44	30·95	55·36	0·31	0·40	0·021	1·54	0·258	0·533
2021	Old leaves	20.4.28	8·65	2·34	2·32	31·48	55·21	0·56	0·43	0·018	0·51	0·118	0·258
2044	Old leaves	21.5.28	7·96	2·31	2·69	30·65	56·99	0·64	0·37	—	—	0·083	0·192
2047	Old leaves	20.6.28	8·29	2·26	2·26	33·58	53·61	0·56	0·44	0·006	0·58	0·082	0·149
2057	Old leaves	20.7.28	9·44	2·14	2·02	29·69	56·71	0·53	0·40	0·002	0·47	0·082	0·142

TABLE 2.—ASH AND PROTEIN CONTENT OF DIFFERENT ERMELO GRASSES IN PERCENTAGE OF STOVE DRY MATTER.

No.	Plant and Camp.		Date of Sampling.	Organ.	Ash.	Crude Protein.	Crude Fibre.	CaO.	MgO.	Na_2O.	K_2O.	P_2O_5.	Cl.
1	*Themeda triandra*.....	O	27.4.26	Green leaves...	—	4·9	29·71	0·30	0·33	0·007	0·80	0·183	—
95	,, ,, ...	O	21.8.26	Brown leaves..	10·88	2·6	—	0·35	0·23	—	—	0·084	0·088
1925	,, ,, ...	O	3.2.28	Green leaves...	9·7	5·74	—	0·42	0·23	0·044	1·98	0·264	0·286
2025	,, ,, ...	O	26.4.28	Green leaves...	7·87	5·9	—	0·47	0·30	0·044	1·98	0·404	0·540
2063	,, ,, ...	O	30.7.28	Brown leaves..	9·31	2·78	—	0·36	0·25	Trace	—	0·159	0·000
2072	,, ,, ...	O	2.11.28	Green leaves...	8·84	8·57	—	0·51	0·37	0·008	1·42	0·388	0·682
4	*Themeda triandra*.....	Q	27.4.26	Mixed leaves...	—	3·3	32·0	0·30	0·39	Trace	0·66	0·144	—
73	,, ,, ...	Q	14.8.26	Brown leaves..	8·50	—	—	0·37	0·22	—	—	0·145	—
247	,, ,, ...	Q	5.10.26	Green leaves...	9·48	—	—	0·43	0·32	—	—	0·548	0·906
479	,, ,, ...	Q	17.11.26	Green leaves...	10·24	9·10	28·26	0·37	0·27	0·0	1·31	0·354	0·703
2029	,, ,, ...	Q	3.5.28	Green leaves...	9·06	—	—	0·49	0·25	—	—	0·280	0·443
2066	,, ,, ...	Q	31.7.28	Brown leaves..	9·55	—	—	0·44	0·24	—	—	0·139	0·187
2	*Themeda triandra*.....	H	27.4.26	Mixed leaves...	—	4·59	28·4	0·46	0·37	Trace	0·57	0·135	—
1914	,, ,, ...	H	30.1.28	Green leaves...	8·52	5·54	—	0·49	0·24	—	—	0·287	0·320
2026	,, ,, ...	H	28.4.28	Green leaves...	8·27	5·48	—	0·43	0·25	—	—	0·224	0·471
2062	,, ,, ...	H	25.7.28	Old leaves.....	9·19	2·14	—	0·46	0·13	—	—	0·069	0·208
2073	,, ,, ...	H	2.11.28	Green leaves...	7·65	9·49	—	0·58	0·34	0·02	1·48	0·456	0·760
136	*Hyparrhenia Buchanani*	O	7.9.26	Green leaves...	7·31	9·38	—	0·54	0·55	—	—	0·504	0·717
248	,, ,,	O	5.10.26	Green leaves...	8·86	—	—	0·67	0·66	Trace	1·54	0·512	—
1452	,, ,,	O	22.6.27	Brown leaves..	9·44	2·27	—	0·49	0·51	—	—	0·080	0·301
1913	,, ,,	O	21.1.28	Green leaves...	8·78	6·65	—	0·48	0·54	0·015	1·18	0·246	0·319
2024	,, ,,	O	26.4.28	Green leaves...	9·57	5·70	—	0·71	0·44	0·020	0·63	0·252	0·119
2060	,, ,,	O	25.7.28	Green leaves...	8·45	5·66	—	0·80	0·57	Trace	0·64	0·320	0·553
2061	,, ,,	O	25.7.28	Brown leaves..	8·21	2·50	—	0·79	0·47	0·009	0·28	0·159	0·139
2071	,, ,,	O	2.11.28	Green leaves...	7·91	7·95	—	0·50	0·67	0·009	1·18	0·304	0·660
859	*Monocymbium cerisae-forme*.....	B	2.2.27	Young haulms.	—	—	—	0·14	0·19	0·005	1·08	0·189	—
860	,, ,,	B	2.2.27	Green leaves...	7·64	—	—	0·35	0·22	—	—	0·159	0·303
1128	,, ,,	B	30.3.27	Green leaves...	—	—	—	—	—	0·004	0·64	0·133	—
1373	,, ,,	B	31.5.27	Brown leaves..	7·59	—	—	0·38	0·21	—	—	0·071	0·149
1572a	,, ,,	B	1.8.27	Brown leaves..	6·66	—	—	0·33	0·16	0·057	0·051	0·045	0·177
1822	,, ,,	B	4.10.27	Brown leaves..	7·20	—	—	0·29	0·16	—	—	0·056	0·142

No.	Plant and Camp.		Date of Sampling.	Organ.	Ash.	Crude Protein.	Crude Fibre.	CaO.	MgO.	Na_2O.	K_2O.	P_2O_5.	Cl.
1864	*Monocymbium cerisaeforme*.....	B	30.11.27	Green leaves...	7·16	—	—	0·22	0·25	0·035	1·16	0·260	0·447
1882	,, ,	B	27.12.27	Green leaves...	5·55	6·43	—	0·25	0·24	—	—	0·194	0·291
1916	, ,,	B	31.1.28	Green leaves...	5·86	—	—	0·23	0·23	0·070	0·82	0·197	0·295
1974	,, .	B	29.2.28	Green leaves...	6·43	—	—	0·30	0·19	—	—	0·172	0·068
1926	*Cynodon Dactylon*....	Q	4.2.28	Green leaves...	8·09	9·46	—	0·50	0·27	—	—	0·319	0·355
2027	,, ,, ...	Q	30.4.28	Mixed leaves...	11·13	7·25	—	0·53	0·35	—	—	0·232	0·391
2065	,, ,, ...	Q	31.7.28	Mixed leaves...	11·56	4·19	—	0·28	0·22	—	—	0·141	0·497
2075	,, ,, ...	Q	2.11.28	Green leaves...	10·03	18·99	—	0·62	0·49	0·09	1·35	0·580	0·672
1a	*Eragrostis spec*.....	T	27.4.26	Green leaves...	—	—	—	0·37	0·09	0·02	0·75	0·217	—
1927	,, ,, ...	T	4.2.28	Green leaves...	5·41	—	—	0·36	0·21	—	—	0·324	0·241
2028	,, ,, ...	T	30.4.28	Green leaves...	5·04	—	—	0·25	0·16	—	—	0·336	—
2064	,, ,, ...	T	30.7.28	Old leaves.....	5·14	—	—	0·26	0·16	—	—	0·143	0·187
2074	,, ,, ...	T	2.11.28	Green leaves...	5·72	8·92	—	0·31	0·19	0·008	1·32	0·332	0·551
1209	*Panicum laevifolium*..		–.4.27	Hay, mixed....	—	6·38	—	—	—	0·104	—	0·111	0·582
961	Mixed grass.....	E	28.2.27	Mixed leaves...	—	—	—	—	—	0·03	0·91	0·250	0·312
—	*Eragrostis Abyssinica*.		–.2.26	Hay.....	—	—	—	—	—	0·02	1·27	—	—
—	,, ,, ..		–.3.26	Hay.....	—	—	—	—	—	0·011	1·27	—	—
1208	,, ,, ..		–.4.27	Hay.....	—	8·53	—	—	—	0·011	1·31	—	0·208
556	Mixed grass.....	B	2.12.26	Mixed green leaves.....	—	—	—	0·34	0·24	0·03	1·23	0·264	0·344
557	,, ,, ...	B	2.12.26	Brown leaves..	—	—	—	0·22	0·14	—	—	0·087	0·080
791	,, ,, ...	B	22.1.27	Mixed leaves...	—	—	—	0·28	0·25	0·05	1·67	0·169	0·355
480	*Harpechloa capensis*...	B	17.11.26	Green leaves...	—	—	—	0·39	0·29	0·04	1·35	0·260	0·746
555	,, ,, ...	B	1.12.26	Green leaves...	—	—	—	0·29	0·29	0·05	1·42	0·282	0·622

Table 3.—Ermelo.

Sodium and Potassium Analyses of Maize. Not Fertilized. In Percentage of Dry Matter.

No.	Organ.	Date.	Na_2O.	K_2O.
592	Leaves.....	13.12.26	0·04	2·26
858	♂Spikes.....	1.2.27	—	1·97
958	Leaves.....	1.2.27	0·06	2·53
959	Stalks.....	1.2.27	0·093	3·16
962	♂Spikes.....	28.2.27	—	1·45
964	Centres, leaves, round cob	28.2.27	Trace	0·95
966	Green leaves.....	28.2.27	0·14	2·50
1123	Green leaves.....	30.3.27	0·17	2·50

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The Phosphorus Content of Grasses in the Eastern Transvaal.

By M. HENRICI, Ph.D., D.Sc., Plant Physiologist, Division of Plant Industry.

The following investigation was undertaken with the purpose of comparing the phosphorus content of grasses on a soil poor in phosphorus but under higher rainfall with that of grasses in Bechuanaland, on a soil poor in phosphorus and under a low rainfall. The Government farm Nooitgedacht 10 belonging to the Division of Veterinary Services, 2½ miles outside Ermelo, Transvaal, proved to be suitable for the investigation as it showed the different varieties of soils occurring in the Highveld of the Eastern Transvaal. The farm is divided into different camps, in which red sand, black loam, and a grey sandy soil are found. The main collection of grasses was therefore made in four camps, viz.: on red sand in O camp, on black loam in H camp, and on grey sandy soil in Q and B camps. Some patches of black loam different from that of H camp were present in B camp and some grass samples were taken from these patches, which were, with regard to the vegetation decidely sour veld, the soil being very wet. The two camps were chosen on the grey soil, because to the eye of the botanist, the vegetation differed in some parts in the two places; B camp, in spite of having a great variety of grasses, made rather a poor impression and Q camp, being a uniform *Themeda* veld, had few other grasses in it. The grass vegetation on the various other soils also differed somewhat, the black loam carrying a pure *Themeda* veld, while on the red sand *Themeda* was dominant with a number of other grasses, e.g. *Hyparrhenia* and *Cymbopogon* intermixed. *Themeda triandra* is the characteristic grass of this part of the Transvaal; it appears that a very mixed grass vegetation, with little *Themeda*, occurs only on poorer soil.

It was thought interesting to collect the same species from all of the different soils. Thus *Themeda triandra* was taken from each of the camps, as well as from a fire-break to see whether burning of the veld had any influence on the phosphorus content of this grass.

The other grasses sampled were *Hyparrhenia buchanani*, from the red soil of O camp, and *Pennisetum thunbergii*, Kunth., *Harpechloa capensis*, *Digitaria tricholaenoides*, *Monocymbium cerisaeforme*, and an *Eragrostis* sp. from the grey soil of B camp. *Arundinella Ecklonii*, Nees., and *Setaria flabellata*, R. and S., were collected at infrequent intervals from the black soil patches of B camp. *Cynodon dactylon*, growing on the banks of a spruit, was taken as a representative of the grey soil of Q camp.

During the summer months the grasses were collected about every tenth day and in winter after longer intervals. The different parts, green and brown leaves, haulms, spikes, roots and rootneck were analysed separately. Young leaves were not analysed separately, as, unlike in Bechuanaland, they are produced only in spring in sufficient quantity for analysis.

SOILS.

My acknowledgments are due to the Division of Chemistry, Pretoria, for doing the analysis of the soils. As may be seen from the attached Table 1, all the soils are very poor in available phosphorus.

TABLE 1.

Soils of Nooitgedacht 10, *Ermelo District, Transvaal.*

	Grey Soil.	Dark Brown Soil.	Black Soil.	Grey Soil.	Red Sandy Soil.
	Camp B.	Camp B.	Camp H.	Camp Q.	Camp O.
Moisture	0·46	0·91	6·42	0·74	1·86
Loss on Ignition	2·67	4·77	5·79	2·04	5·15
Insoluble Matter	91·30	87·00	73·18	94·37	75·80
Iron and Aluminium Oxide	1·84	1·8	—	—	—
	2·90	4·63	12·84	2·52	15·52
CaO	0·05	0·06	0·70	0·06	0·07
MgO	0·08	0·08	0·82	0·07	0·14
K_2O	0·15	0·27	0·12	0·03	0·12
P_2O_5	0·03	0·03	0·03	0·03	0·07
Soluble Silica	0·23	0·22	0·31	0·09	1·08
Nitrogen	0·078	0·193	0·084	0·056	0·090
Available K_2O	0·012	0·007	0·0035	0·0128	0·0131
Available P_2O_5	0·001	0·002	0·0012	0·0014	0·0007
Available CaO	0·010	0·016	0·326	0·023	0·046
Available MgO	0·009	0·010	0·152	0·014	0·027
PH Value	5·2	5·1	—	—	—

The available phosphorus is about the same as, perhaps rather more, than in the Bechuanaland soils (Henrici, 1928).

As it varies so little in the five Ermelo soils, the differences in the phosphorus content of the various grasses cannot be attributed solely to variations in the available soil P_2O_5.

As to the other minerals, iron and aluminium are abundant in the red sandy soils and in the black soils; calcium and magnesium (both also in the available form) are abundant in the black " vlei " soil alone, all the other soils being poor in these minerals.

RAINFALL.

The figures for the rainfall were obtained from the adjoining farm, belonging to the Forestry Department. The Forestry farm is a plantation, whilst the farm Nooitgedacht 10 is the natural grass

veld, grazed down regularly by sheep or cattle. In the hilly part of the farm shrubs and bushes are interspersed with grasses, this being especially the case on the red sandy soil. Possibly owing to its being a plantation the rainfall of the Forestry farm is slightly higher than that of Nooitgedacht 10. But no other figures are available.

The rainfall is given for the period during which the phosphorus investigation was carried out, viz., July, 1926 to December, 1927.

Rainfall of Nooitgedacht 10, *Ermelo District, Transvaal, in cm.*

	July	Aug	Sept.	Oct.	Nov.	Dec.
1926.....	0·21	—	10·4	2·7	8·31	11·12

	Jan.	Feb.	March.	April.	May.	June.
1927.....	11·18	7·95	7·7	3·32	2·31	—

	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1927.....	6·34	—	2·6	16·47	4·68	9·62

The rainfall from July, 1926, to June, 1927, was 65.20 cm. and from September, 1926, to August, 1927, was 71.33 cm.

As can be seen from these figures the rainfall is about twice that of Armoedsvlakte, Bechuanaland, where the first phosphorus determinations were made (Henrici, 1928). The soil at Ermelo was consequently never as dry as at Armoedsvlakte, and neither temperature nor evaporation was so extreme. As a matter of fact it was often misty in Ermelo even on days when it was not raining. Under these conditions wilting was seldom seen in Ermelo in the year 1926-27; this does not mean, of course, that wilting does not occur there during drier seasons. The phosphorus determinations were made in a year over which the distribution of rain was very even. This makes it more interesting for comparison with Bechuanaland.

METHOD OF ANALYSIS.

The same micro method of Atkins (1924) in the modification previously described (Henrici, 1928) was used. In the course of the determinations some further modifications proved to be useful. As the phosphorus content was rather high in some cases it was necessary

to add more reducing agent without altering the acidity. This was easily done by making the stannous chloride solution double the strength without adding more hydrochloric acid, that is to say, 0.2 gm. of tin was dissolved in 2 c.c. HCl., 4 drops of copper sulphate added and the whole made up to 10 c.c. Tests were made with this reagent to make sure that it produced the colour quantitatively.

A new Bausch and Lomb, 1927 model, colorimeter was used. As far as possible readings were taken over the range 20-30 mm. on the colorimeter scale, the standard (see Henrici, 1928) being set at 30 mm. Experience showed that readings under 15 should be regarded as unreliable, while those over 35 became too faint for accurate reading.

Again, as far as possible, .1 or .2 gm. of material was taken for an analysis. In the Armoedsvlakte investigation, .3 gm. (old material) gave quite satisfactory results, but with the Ermelo material, and the new reagent B (stannous chloride) good duplicate results were not always obtained when more than .2 gm. of the material was used.

As the young leaves, spikes, etc., often contained large amounts of phosphorus, it became necessary to use less than .1 gm. of material in order to obtain a workable colorimeter reading. To avoid the errors introduced in weighing out very small quantities, .1 gm. or .2 gm. was made up to 100 c.c. solution and suitable dilutions made for individual determinations.

The dilution had to be done before the addition of the reducing agent; dilution after the blue colour had developed caused the solution to become cloudy.

This is a disadvantage of the method, which is avoided in Fiske and Subbarow's modification (1925), in which a much less acid solution is used, with Eikonogen as the reducing agent.

A second ashing with nitric acid proved unnecessary. Ashing was done with a few drops of 10 per cent. calcium acetate.

The material used for the analysis was ground into a fine powder with Alexander mills. The need for this was shown by a test on comparatively roughly ground grass, .1 gm. being taken for each test.

Coarsely ground sample, average .150 per cent. P_2O_5.
Fine portion sifted out ... ... .184 per cent. P_2O_5.
Coarse portion sifted out ... ... .131 per cent. P_2O_5.

All analyses were done in duplicate, often in triplicate when unexpected results were obtained. The duplicates were not done at the same time, to avoid the error which may occur in colorimetric methods that material treated in exactly the same way give good duplicates but not necessarily accurate results.

In the literature (Echevin, 1926) it is emphasized that the calculation of the mineral values should be done on equal surface. This is, however, impossible for values calculated on a whole plant, and for the present the phosphorus has to be expressed as a percentage of the dry matter. At nearly every sampling a whole tuft of each grass was pulled to pieces and the total weight of roots, rootneck, green

and brown leaves, green and brown haulms and spikes was determined. Then the relation of all the organs to the weight of the root was calculated, the root being taken as 1. By this means the phosphorus could be given in absolute figures as well as in percentages. A plant corresponding to 100 gm. roots was constructed, the phosphorus of the other organs being then calculated from the percentage figure of the organ to the root. By addition the total amount of P_2O_5 in a plant corresponding to 100 gm. roots or the total amount in the aerial parts of a plant corresponding to 100 gm. roots may be obtained. Needless to say, when working with plants in the open veld, and not with pot plants, the method cannot give quite accurate figures, as tufts in free nature vary in size and age, but in spite of this they give quite a good idea of the amount of P_2O_5 present in the plant, of the distribution of the phosphorus during the year and very valuable information on the re-migration of P_2O_5 in autumn and during a drought. They also offer a good comparison for the phosphorus content of grasses in differing areas. Great care was taken to collect plants of about the same size.

From veld observation it was obvious that some grasses of the Eastern Transvaal produced seeds—the conditions were apparently not quite as bad as in Bechuanaland as it was possible to grow maize on unfertilized virgin soil near B camp—and it was thought interesting to compare (*a*) the phosphorus content of maize with that of the veld grasses and with maize grown on fertilized soil and (*b*) to see if some compounds of phosphorus were present in equal amounts in maize as in veld grass.

The following compounds were determined:—

The phosphorus of the nucleoproteids by the method of Plimmer. (1906). As the original literature was not all at my disposal when the analyses were made the methods given in the handbook of Kostytschew (1926) were used with modifications to suit the outfit of the laboratory.

For the precipitation of the nucleoproteids, 2 gm. of finely ground material were digested with 1 per cent. NaOH at 37° C. for two days. The solution was filtered and the filtrate neutralised with 0.2 per cent alcoholic HCl and slightly acidified. The nucleoproteids came down on standing. If the precipitation was done at room temperature, it often happened that the nucleoproteids were not completely precipitated, but that part of them remained in colloidal solution, which could be cleared by warming on a water-bath. This heating curtailed the subsequent filtration which was done through an ash-free filter paper, with suction. The dried precipitate and filter paper were ashed and the ash treated for P_2O_5 as described above. Generally the final solution of the nucleoproteids had to be diluted to four times the original volume, this could easily be estimated in a preliminary determination.

The phosphorus of the Lecithin was determined by extracting 10 gm. of material with alcohol and ether. Unfortunately no Soxhlet Apparatus was available, and the extraction had therefore to be extended for 12 hours. After filtering, the alcohol-ether extract was evaporated to dryness and ashed for microanalysis. Great care has to be taken that the ether extract does not creep over the crucible, during evaporation on the water-bath. The amount of phosphorus

from the Lecithin was very small. Often no dilution was necessary at the end.

The water soluble phosphorus was extracted from 1-2 gm. material by steaming it for about six hours on a water-bath. After filtering the solution was slightly acidified, which brought down a lot of organic material on evaporating; all the phosphorus, however, remained in solution. Tests were carried out to make sure of this point. After filtering, the phosphorus was precipitated with Magnesia mixture on warming (Treadwell, 1924). At first the precipitate was re-dissolved in HCl and precipitated again with Magnesia mixture. The precipitate was then filtered through ash-free paper, ashed and the phosphorus determined. As the Magnesium ammonium phosphate was not determined as such, the double precipitation seemed unnecessary and the first precipitate was filtered and ashed.

The determination of phytin offered some difficulties. If the phytin was determined in an aqueous extract it proved to be a very small quantity. Tests were done on pure phytin with the method of Henbner (1914), the phytin used being the product of Hoffman La Roche, Basle. If phytin was dissolved in pure water only 30 per cent. of the real phosphorus value, was recovered, but if dissolved in 0.6 per cent. HCl, the right percentage of phosphorus was obtained. Accordingly the plant material was extracted with 0.6 per cent. HCl and titrated in this solution, the value being about three times as high as that for the water extract. To the solution 3 c.c. of a 0.03 per cent. solution of Ammonium rhodanide was added as indicator. A standardized solution of chloride of iron, 0.05 per cent. Fe, was added from a burette until the solution assumed a permanent red tint. If much phytin was present, a white precipitate came down when about half the amount of chloride of iron had been added. With small quantities of phytin no precipitate came down, but a slight cloudiness was observed. As the plant extract was coloured and the titration is based on the appearance of a red colour the change of colour could not easily be detected. Addition of ether to the extract towards the end of the titration overcame this difficulty. Ammonium rhodanide and iron rhodanide are both easily soluble in the ether layer, and the colour change can easily be noted. During the titration the flask must be well shaken, so that the added iron comes in contact with the phytin in the aqueous layer, and does not merely react with the rhodanide in the ether layer. At first a pink colour appears in the ether layer, which disappears immediately on shaking; when the colour persists for five minutes, the reaction is complete.

It seems advisable to add the ether as near the end of the titration as possible.

1 mg. of the standardized iron-chloride corresponds to 1.19 mg. P. As the phytin in the plant is only partly soluble in water, it is not possible to calculate directly from the water soluble phosphorus by subtraction the amount of phosphorus in form of glycero-phosphoric acids and sugar phosphoric acids, as was suggested by Kostytschew (1926.) This value can be derived approximately only by taking the third part of the value of phytin as basis.

The determination of the phytin in water extract was done only in a limited number of analyses. It appeared to the author that less of the total phytin was soluble in the older leaves than in the young ones.

The author is very much indebted to Mr. P. Potter for his assistance in the analytical work, especially with the determinations of the total phosphorus content. Without his most valuable help it would not have been possible to finish the analyses in the time allowed for this work. The determination of the grasses was kindly done by Miss Sydney Stent to whom my heartiest thanks are due.

RESULTS.

(*a*) Phosphorus Content in Percentage.

(1) *Leaves.*

The figures for phosphorus calculated as percentage P_2O_5 in the dry matter are given in the Tables 2-15. The phosphorus content of green leaves may be discussed first. A glance at the tables reveals the fact that, with the exception of young Spring leaves, high phosphorus values do not occur. It will further be noted that the phosphorus content of the different species on the same soil varies, and that the values of the same species on different soils vary as well. The latter point is illustrated in Table 16 which gives the monthly average value of *Themeda triandra* in the different camps.

Table 16.

Themeda triandra. Monthly average value of P_2O_5 in green leaves.

Camp.	1926.				
	Aug.	Sept.	Oct.	Nov.	Dec.
O. Camp, no leaves.....	—	0·40	0·38	0·26	0·22
H. Camp, ,, ...	—	0·45*	0·24	0·22	0·20‡
B. Camp, ,, ...	—	0·40	0·33	0·19	0·21
Q. Camp, ,, ...	—	0·52	0·48	0·32	0·25
Q. Camp, ,, fire break...	—	0·40	0·29	0·31	0·23

* Only one determination—Beginning of the month, no green leaves.
† Only one determination—End of month no green leaves.
‡ Relative Minimum.
§ Absolute Minimum.

Camp.	1927.								
	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.
O. Camp, no leaves.....	0·21	0·20§	0·22	0·27	0·25	0·25*	0·31	0·52	0·63
H. Camp, ,, ...	0·20‡	0·21	0·22	0·19	0·14§	0·16	0·16	0·46*	0·55
B. Camp, ,, ...	0·19	0·19	0·17‡	0·18	0·12§	0·18	—	0·45*	0·44
Q. Camp, ,, ...	0·22	0·22	0·21	0·20	0·16§	0·42	—	0·36	0·49
Q. Camp, no leaves, fire break	0·17§	0·18	0·23	0·36	0·30	0·28	—	—	0·62

* Only one determination—Beginning of the month no green leaves.
† Only one determination—End of month no green leaves.
‡ Relative Minimum.
§ Absolute Minimum.

From table 16 the difference between the two winters is obvious. In 1926 green leaves of *Themeda* were first found in September, owing to the heavy frosts and the drought in winter; this means that for nearly four months no green leaves were available. A collection at the end of April, 1926 showed very few green leaves. Winter in 1927 was quite different, by no means as cold as, and less dry than that of 1926. The result was that in two camps there were green leaves, although only in small quantities, throughout the winter; in the other camps they were lacking only for about a month. Another interesting point was that after the milder winter, in four cases out of five the Spring values were considerably higher than those after the severe 1926 winter. The youngest leaves which appeared in August were not the richest in phosphorus, the September leaves claimed this place. This was rather peculiar because it could not have been due to lack of water—in July 6.34 cm. rain fell and in September only 2.6—but it was a fact which was observed also in Bechuanaland (Henrici, 1928.) The highest and the lowest values in the different camps varied from camp to camp. Whilst the maxima of phosphorus content in the various camps in the same season differed by about 40 per cent., the minima differed by about 66 per cent. O camp which had the highest phosphorus figures, showed also the highest absolute minimum; B camp with the lowest maximum of phosphorus showed also the lowest minimum. The change from the high values to the lower values was different in the various camps. The phosphorus contents dropped most quickly in H camp and most slowly in O camp. This was not accidental, the heavy clay soil of H camp, by inhibiting, the free movement of water, tends to withhold from the plant the small amount of available P that is in the soil, while the light sandy soils of O and Q camps allow the plant to utilize the last traces of water. Q camp was especially favoured by nature in that the spruit at its lowest part, where *Cynodon* was collected, kept the soil always moist. Not only do the minima of the phosphorus content differ in the various camps, but they also showed a drop in different months. The winter minimum and the summer minimum had to be distinguished from each other. There was no pronounced time of summer minimum as there was at Armoedsvlakte (Bechuanaland), H camp as the driest camp physiologically speaking, had a summer minimum from December to January, at the time at which it was also observed in Bechuanaland: O camp had it only in February, in B camp it occurred as late as March (at this time it appeared as though B camp received much less rain than the other parts of the farm). Q camp, the wettest camp, had no summer minimum whatsoever. This created the impression that climatic conditions had a lot to do with the summer minimum. The more soil moisture available, the less marked is a summer minimum.

With the exception of O camp the winter minimum was an absolute one, but as there were no green leaves for about three weeks in June in this camp, the exception was only apparent; a minimum would have occurred just before the green leaves disappeared. The absolute winter minimum was considered important in order to characterize the veld from the point of view of phosphorus. It means that the winter veld is always worse than the dry summer veld; in Bechuanaland this was not the case.

From all the data, it appears that as far as *Themeda* is concerned O camp is the best and B camp the worst. Compared with the Bechuanaland analyses the young spring leaves of *Themeda* in the Ermelo District are richer in phosphorus, but the spring leaves are very few and soon afterwards the phosphorus content of the leaves falls to the same level as that of leaves from Vryburg, and the summer minima are even lower. No explanation can be offered as to the cause of this fact; it only confirms the practical experience that the winter veld in the Highveld near Ermelo is worse for cattle than the winter veld in Bechuanaland. Cattle can scarcely survive in Ermelo without the addition of extra rations to their grazing.

From the information thus obtained for the grasses on the different soils it is concluded that for winter grazing it is advisable to avoid areas such as B or H camps, which are decidedly poorer in winter than hill or vlei camps, such as O and Q.

As was mentioned in the introduction, samples of *Themeda* were collected from a firebreak in order to compare their phosphorus content with that of the ordinary veld plant. It appears that the phosphorus content of the leaves in the beginning of the season is rather lower than that in the plant on the unburnt veld; only in the second half of the season, after plenty of rain, are the plants on the firebreak at a strong advantage.

TABLE 17. GREEN LEAVES.—P_2O_5 AS PERCENTAGE OF DRY MATTER. AVERAGE VALUES.

Camp.	1926.						1927.								
	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.
Q.—*Cynodon Dactylon*	0·27	0·28	0·46	0·35	0·28	0·29	0·26	‡0·22	0·28	0·32	0·51	0·32	*	*	0·98
O.—*Hyparrhenia Buchanani*	‡0·16	0·38	0·40	0·26	0·25	0·22	0·18	†0·17	0·26	0·28	0·30	0·32	0·35	0·44	0·55
B.—*Pennisetum Thunbergii*	*	*	0·50	0·25	0·29	0·28	†0·20	0·23	0·25	0·25	†0·20	0·35	0·25	0·75	0·72
B.—*Digitaria tricholaenoides*	*	*	*	0·23	0·18	0·17	†0·15	0·28	0·17	0·19	*	‡0·12	*	*	0·42
B.—*Monocymbium cerisaeforme*	*	*	0·42	0·32	0·25	0·24	0·15	0·15	‡0·12	0·15	0·32	*	*	*	0·50
B.—*Harpechloa capensis*	*	*	*	0·16	0·16	0·18	0·14	0·18	†0·11	0·13	‡0·09	0·12	*	0·26	0·32
B.—*Eragrostis*, spec.	*	0·25	0·42	0·25	0·26	0·24	‡0·21	0·27	0·34	0·31	0·26	†0·22	0·23	0·23	0·41
B.—*Arundinella Ecklonii*	*	—	—	0·27	0·23	0·18	†0·17	†0·17	0·22	—	‡0·12	0·30	—	—	—
B.—*Setaria Imberbis*	0·18	—	—	0·23	0·22	0·18	0·14	*	*	*	—	—	—	—	—

* No green leaves found.
† Relative Minimum.
‡ Absolute Minimum.

Blank Space = not collected.

After the explanation offered for *Themeda* on different soils the interpretation of Table 17, which is a summary of Tables 2-15, is not difficult. There is no doubt, that O and Q camps are much better than B camp. There are some good grasses in B camp like *Pennisetum thunbergii* and *Eragrostis*, but these grasses are so scarce that in considering the grazing bulk they scarcely come into consideration. Where *Pennisetum* appeared, it was so grazed and tramped down by the cattle that it was in danger of disappearing entirely. *Digitaria tricholaenoides* which is a very palatable grass proved from the point of view of phosphorus to be rather disappointing. *Setaria imberbis* grew for a period of 5 months only in the season 1926-27. *Harpechloa capensis* and *Monocymbium cerisaeforme* (one of the dominating grasses of B camp), were really the worst grasses from the point of view of phosphorus. *Monocymbium* was eaten only when it was young, and for a long time it had no green leaves at all.

The result obtained for the other grasses are very similar to those obtained for *Themeda*. If green leaves are present at all in the winter the absolute minimum of phosphorus (with the exception of some good grasses) falls at this time of the year. *Pennisetum*, the one good grass, has a very high minimum. *Eragrostis*, another good grass, has also a high winter minimum—as the detailed tables show, it has its lowest values in the winter. *Cynodon dactylon*, a grass with an absolute minimum in summer, has no green leaves in winter. For a period of two months in winter the minima for several grasses are lower than the minima for the worst grasses in Bechuanaland. The maxima for the good grasses on the other hand are higher than the highest values in Bechuanaland, but as they last only a short time, they do not make good for the average low value. What accentuates the difference in disfavour of the Highveld is the fact that there are no green leaves at all for several months. If the percentage value were the sole deciding factor there would be no doubt that from the point of view of phosphorus, Bechuanaland is the better pasture; but the absolute amount of both grass and phosphorus has also to be taken into consideration before a definite conclusion in this connection may be reached.

From Table 17 it appears that *Cynodon dactylon* is a very good grass, having a high maximum as well as a high minimum. It is eagerly grazed during the whole year, but especially in early spring. *Hyparrhenia* has the advantages of having green leaves all the year round. It is rather a late grass and its old leaves are not very palatable. The fact remains that in spite of having a few good grasses, B camp can be considered as the worst of the camps investigated. On the other hand it is interesting to note that even on this bad veld some grasses are able to take up more phosphorus than the average grass. From the observation that a relative minimum of phosphorus exists in many cases but not at a fixed period like in Bechuanaland, it may be concluded that moisture conditions are an influencing factor, but not the only one causing this minimum, because in none of the summer months was there a severe drought in Ermelo, the only dry period being the first half of March (and then nothing to compare with the conditions in Bechuanaland).

Another point may be mentioned. In every curve for the annual phosphorus content of South African grasses, the high values in

spring for green leaves will be noticed (see Theiler, Green and du Toit, 1921 and 1924, and Henrici, 1928). How does it come about that only at this time of the year the young leaves are able to procure this high amount of phosphorus? In Bechuanaland nearly all grasses had young leaves all the year round, except of course in time of severe drought. The data obtained showed that their phosphorus content was mostly, but not always, higher than that of simultaneously collected old leaves, but never so high as in young spring leaves. In Ermelo this problem is more difficult to investigate. Young leaves do not appear at all times of the season. On the contrary, it was very seldom possible to separate old and young green leaves, as there were none of the latter. When young leaves had to be collected for the special purpose of elucidating this point, it took several hours to collect enough material for a micro-analysis. The following table 18 illustrates the results.

TABLE 18.

P_2O_5, as percentage of dry matter, in young green leaves in Spring and in Autumn.

Species.	Spring.			Autumn.	
	Date.	P_2O_5.	Date.	P_2O_5.	P_2O_5.
				Young Leaves.	Old Leaves.
Panicum laevifolium	5. 9 .27	0·67	4. 3 .28	0·32	0·33
Brachiaria serrata	5. 9 .27	0·58	27. 2 .28	0·33	0·29
Cynodon Bradleyi	5. 9 .27	0·52	27. 2 .28	0·48	0·40
Themeda triandra	27. 9 .26	0·45	27. 2 .28	0·29	0·22
Monocymbium cerisaeforme	28. 9 .26	0·48	27. 2 .28	0·22	0·20
Hyparrhenia Buchanani	30. 9 .27	0·95	27. 2 .28	0·35	0·40
Digitaria tricholaenoides	11.10.26	0·23	29. 2 .28	0·31	0·20
Eragrostis	5. 9 .27	0·54	—	—	—

It appears that all the grasses have higher values for young spring leaves than for young autumn leaves, with the exception of *Digitaria*. If the cause of this phenomenon is sought it must first be borne in mind that in spring there is more phosphorus in the vicinity of the plant. In the course of this investigation it will be seen that not all the phosphorus re-migrating from the leaves is stored in the roots, but part of it is doubtless returned to the soil (Ossipowa and Interewa, 1926, Hansteen Cranner, 1922, Grafe and Magistris, 1926, Melin, 1924), as the absolute amount of phosphorus during winter is much smaller than in summer, in spite of no leaves having fallen off (see page 453). Another explanation cannot be offered. Thus, although it is not possible to show analytically the change of phosphorus in the soil, it has to be accepted that the immediate surroundings of the plant roots are richer in phosphorus in spring

than the general analysis shows. It is, of course, possible that, during winter, this phosphorus changes into a more insoluble form and is only available for plants after the first rains, but nevertheless it seems as though the plant has more phosphorus at its disposal in spring than later in the year. The breaking off of the old leaves occurs much later than winter. In Ermelo old leaves of the previous year's growth often remain on the tuft till December. It is not very likely that they increase the phosphorus content of the soil to any extent, as in winter they are so poor in phosphorus that by the time they decay very little remains to enrich the soil. It is most likely that they are leached out while still on the plant.

One point which has to be emphasized especially is the variability of the phosphorus values of the leaves. It is evident that plants collected in the open veld differ in their phosphorus content to a greater extent than when cultivated in plots although the veld offers a very uniform aspect. On the whole the variations in Ermelo were smaller than in Bechuanaland, probably due to the fact that in the Eastern Transvaal (Ermelo) the veld is better overgrown and more uniform and therefore the collecting could be done on a smaller area. Nevertheless it happened twice even in Ermelo, that a plant was found which had almost double the amount of phosphorus in the leaves, as well as in every other organ, that the average individual of the species for the soil had. Plants nearby had normal figures. The only explanation offered for this result is that the abnormally rich plant was growing near decaying matter containing abundant available phosphorus and was able to take in more phosphorus.

TABLE 19. *Brown Leaves.*—Phosphorus as percentage of dry matter. Average values.

Species.	1926.						1927.								
	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.
Themeda triandra—O. Camp	—	0·07	0·07	0·08	0·11	0·06	0·09	0·06	0·07	0·07	0·08	0·10	0·13	0·08	0·09
Themeda triandra—Q. Camp	—	0·15	0·12	0·09	0·10	0·09	0·07	0·07	0·08	0·08	0·06	0·15	0·10	0·06	0·06
Themeda triandra—Fire Break	—	—	0·09	0·09	0·11	0·13	0·08	0·06	0·09	0·13	0·13	0·14	0·15	0·08	0·08
Themeda triandra—B. Camp	—	0·07	0·08	0·09	0·05	0·08	0·08	0·08	0·05	0·05	0·06	0·07	0·06	0·07	0·15
Themeda triandra—H. Camp	0·09	0·09	0·08	0·07	0·07	0·07	0·06	0·06	0·08	0·08	0·06	0·08	0·05	0·06	0·06
Cynodon Dactylon—Q. Camp	—	—	—	—	0·20	—	0·10	0·07	0·14	0·10	0·15	0·16	0·11	0·11	0·11
Hyparrhenia Buchanani—O. Camp	0·10	0·09	0·08	0·07	0·07	0·11	0·08	0·06	0·08	0·09	0·06	0·11	0·12	0·11	0·11
Pennisetum Thunbergii—B. Camp	—	—	0·06	0·11	0·08	0·11	0·07	0·07	0·11	0·09	0·09	0·11	0·13	0·10	0·11
Digitaria tricholaenoides—B. Camp	—	—	—	0·05	0·06	0·06	0·07	0·09	0·12	0·09	?	0·07	0·07	0·07	0·05
Monocymbium cerisaeforme—B. Camp	0·05	0·05	0·05	0·04	0·05	—	0·04	0·05	0·04	0·08	0·09	0·11	0·06	0·06	0·07
Harpechlaa capensis—B. Camp	—	—	—	0·02	0·03	0·03	0·04	0·04	0·02	0·03	0·03	0·04	0·04	0·04	0·03
Eragrostis spec.—B. Camp	—	—	0·08	0·07	0·08	0·10	0·11	0·12	0·14	0·16	0·17	0·13	0·13	0·10	0·06
Arundinella Ecklonii—B. Camp	0·06	—	—	0·05	0·05	0·05	0·04	0·04	—	0·04	—	0·06	—	—	—
Setaria imberbis—B. Camp	0·07	—	—	0·06	0·05	0·04	0·04	—	—	—	—	—	—	—	—

The monthly average value of phosphorus for brown leaves (Table 19) confirms the results obtained for green leaves. In B camp there are indeed very poor grasses. The phosphorus content of brown leaves of *Monocymbium* and *Harpechloa* is considerably lower than that of the worst grasses in Bechuanaland. Even *Arundinella* and *Setaria imberbis* are on the average lower than bad Bechuanaland grasses. The *Themeda* of B camp is about the same as in Bechuanaland, whilst the *Themeda* of all the other camps has decidedly higher P_2O_5 values. *Pennisetum thunbergii*, *Cynodon dactylon* and especially *Eragrostis*, surpass in value good Bechuanaland grasses. One point has to be remembered: The relation of brown leaves to green leaves is at any time of the year much higher in Ermelo than in Bechuanaland; the actual figures for brown leaves are only partly available for Bechuanaland, as they were united with brown material derived from stalks, rootneck, etc., when there were few brown leaves. But to the eye of the botanist this difference is obvious; Bechuanaland may have any quantity of wilted green leaves or no leaves at all, but except in winter, not many brown leaves, whilst in the Highveld of the Eastern Transvaal wilting is seldom observed but brown leaves are present in large amounts. It may be emphasized that neither farm was heavily grazed and especially in Bechuanaland the sampling was done in spare camps. In Ermelo, the whole of Q camp, and portions of H and O camps were set aside for sampling; B camp was grazed during autumn and winter by cattle under experiment. Grazing therefore cannot be held to account for the difference in the relative amounts of brown and green leaves in either area.

As the better grasses are so limited in amount, they cannot improve the quality of the grazing as a whole.

It has been seen that the P_2O_5 content of the brown leaves in B camp is very low, and it cannot but be concluded that this type of veld is much poorer than Bechuanaland veld. Judging from the percentage figures the other camps do not look so bad, but here again the absolute amount of phosphorus must finally decide (see page 451).

(2) *Haulms and Spikes.*

Contrary to the case in Bechuanaland, the Ermelo grasses usually produce haulms only once a year. In some cases a few ripe seeds were observed, though the germination was not tried out. It is probable that the seeds are sound as young plants of *Eragrostis* and *Cynodon* were observed. Even in *Themeda* some apparently good seeds were found in May. Some of the Ermelo grasses are very late in producing haulms, above all *Monocymbium cerisaeforme* and *Hyparrhenia Buchanani*.

The first question to be investigated is whether the production of haulms is accompanied by a decrease of phosphorus in the green leaves. Is the minimum in summer caused by the haulm production? This question can be answered in the negative. For nearly all of the grasses the production of haulms occurs much earlier than the summer minimum. Thus it is not likely that phosphorus is withdrawn from the leaves for the formation of haulms. In the second part of this investigation it will be seen why leaves do not diminish

their phosphorus at the time of haulm formation. The question arises if any other organ of the plant decreases its phosphorus during the haulm formation. In about half the investigated plants a decrease of the phosphorus in the root or in the rootneck occurs simultaneously with the formation of haulms, thus it is likely that the two processes are correlated. In some cases a drop in the phosphorus content only takes place when the seeds are beginning to harden.

Eragrostis spec., *Cynodon dactylon*, *Pennisetum thunbergii* and *Hyparrhenia Buchanani* produced seeds in fair amounts, the phosphorus of their spikes reaching over 0.35 per cent. As soon as the seeds or the spikelets have fallen, the phosphorus content of the haulms drops rapidly. *Harpechloa* reaches the low value of 0.02 per cent. Apparently the phosphorus is withdrawn from the drying haulms to a much larger extent than from the leaves, a fact which had already been observed in Bechuanaland.

Sometimes old haulms which have remained on the tuft until the new springs leaves are coming up increase their P_2O_5, e.g. *Cynodon*, and the question arises if this migration of the phosphorus from the old haulms to the root and from the root again into the haulms is not purely a mechanical process, having very little to do with physiology.

(3) *Roots and Rootneck.*

Amongst the investigated grasses are some which utilize the rootneck, others the root as a storage organ for phosphorus. In most cases both organs show a distinct yearly curve of the phosphorus content. Generally speaking the time of active growth coincides with all the low values of the root and rootneck, while in autumn and winter root and rootneck contain large amounts of phosphorus. A single species may differ by a month from another with the beginning of the increase of phosphorus; generally the increase of phosphorus for the root is between April and September. The maximum may fall in June as a real winter maximum, or even as early as April, or it may as well fall in August just before the plant begins to shoot again. The maximum is very variable for the different species and is by no means a criterion of the quality of the grass. *Monoeymbium* has a slightly higher maximum than *Eragrostis*. All *Themeda* samples are characterized by a high maximum, up to four times as high as *Eragrostis*. The minimum value of P_2O_5 in the roots varies much less. With few exceptions it lies between 0.06-0.09 per cent. P_2O_5, the exceptions are good (*Pennisetum*) or bad (*Harpechloa*) grasses, thus again the quality of the grass is independent of the root minimum.

The same holds good for the maximum and minimum in the rootneck. January and February is decidely the time where the least phosphorus is found in the rootneck, the values varying from 0.05-0.11 per cent. The maximum, varying from 0.15-0.49 per cent., lies mostly between June and August, with some exceptions where very high values are found just before haulm formations. Maxima and minima for the root and the rootneck of one species do not always occur at the same time.

There is no doubt that root or rootneck serve as storage places for the phosphorus in autumn and winter. On the other hand the percentage figures alone do not afford sufficient proof that all the re-migrating P_2O_5 of the leaves is stored in the root—only absolute figures can decide this.

(*b*) Absolute Amount of Phosphorus.

From a glance at Tables 20-27 it will be seen that the absolute amount of P_2O_5 in a grass corresponding to 100 gm. root is not constant during the year, neither is it constant in the aerial parts of such a plant; on the contrary, the fluctuations are relatively larger in the latter. The absolute amount of P_2O_5 for different species of grasses varies, although a number of species have about the same amount of phosphorus in the aerial parts. Except for *Cynodon*, in no case does the phosphorus contained in spikes and haulms affect the absolute amount of phosphorus of the aerial parts to any degree. It lies in the course of nature, that the absolute amount of phosphorus for the green leaves varies generally speaking, inversely, as that for the subterranean parts.

The question arises whether the highest values of the total phosphorus of a species coincide with the highest values in the aerial parts. The question can be answered partly in the affirmative. In spring and summer the high values for the total phosphorus of a grass tuft correspond with high values for the aerial parts although the maxima do not necessarily coincide. But in autumn and winter this relation does not exist. For the present discussion *Cynodon dactylon* and *Themeda triandra*, from the firebreak, may be excluded, as they represent special cases. As to the character of the curves very regular and rather irregular ones may be distinguished. The graph for the phosphorus content of *Harpechloa* may be called an ideal curve from the point of view of regularity, but it certainly shows very low values throughout the season. Nowhere is there an accentuated maximum; the total phosphorus of the whole plant and of its aerial parts runs nearly parallel in summer; in winter the difference between the two is slightly larger. The value for the whole plant does not exceed 1.10 gm., and for the aerial parts 0.9 gm. P_2O_5. What does this mean in practice? 100 gm. roots carry very few leaves, that is to say, *Harpechloa* is from the economic point of view of water and salt supply, a very poor grass, the few leaves having such a low phosphorus content, that the grass can be regarded as a sheer waster when it is considered what an area 100 gm. roots—the basis of this calculation—explore for water and minerals. *Harpechloa* is considered the worst grass so far investigated in the Highveld.

Hyparrhenia, *Digitaria*, *Themeda* from B camp and *Monocymbium* also show rather regular curves. First of all, all of them, at at least one period, have a good lot of phosphorus in the plant, either in November or December; *Hyparrhenia* and *Monocymbium* have a second maximum in the late summer. Before the spring maximum there is very often a smaller relative maximum quite early in the

season, in August or September; and this is not considered to be accidental. The explanation may be the following:—

In early spring, when in a normal year water is not scarce in Ermelo, and the whole supply of phosphorus is still around the roots of the plant, enough P_2O_5 can be absorbed to supply the young leaves liberally.

Then before the regular rains begin, elongation and a certain lignification take place, evaporation increases with the advance of the season and the phosphorus near by the plant is exhausted.

New green leaves are still being produced, but all the aerial parts must now be deficient in phosphorus.

Only early grasses coming up before the rains show this spring maximum; the absolute maximum occurs after heavy rains have fallen, i.e. when more phosphorus has been dissolved.

For the grasses mentioned in the above paragraph the absolute amount of P_2O_5 for the whole plant, as well as that for the aerial parts differ considerably. *Hyparrhenia* has values of 3.8 gm. and 2.9 gm. respectively. The values for *Monocymbium* and *Themeda* B camp are ca. 2.0 and 1.8 gm. The relation between the phosphorus in the whole plant and its aerial parts is very different for the four grasses. In *Monocymbium* most of the phosphorus is in the aerial parts, in winter of course less than in summer.

Themeda, B camp, *Hyparrhenia* and *Digitaria* have all more phosphorus in the subterranean parts than *Monocymbium*, the difference in this respect between *Themeda* and *Hyparrhenia* and *Monocymbium*, being most marked during the months March to September.

Digitaria always has a considerable amount of phosphorus in the subterranean parts, and even in summer the curve for the aerial parts never follows closely that for the total in the whole plant.

As this type of curve is met with in other Highveld grasses, it is worth while considering the reasons for it.

The impression is given that *Digitaria* does not thrive under the climatic conditions of the Highveld—probably the soil is too poor.

Digitaria is one of the grasses that grow late in the season; at first it appears to be very palatable and nutritious, but it soon dies after producing very few haulms. It has a very well developed root-neck, and yet the brown leaves soon decay, in fact they are difficult to find at all in winter. In this respect this *Digitaria* is different from its relative, the woolly-finger grass. The old leaves are very poor in minerals.

The curves to be considered now are much more irregular. To what is this due? It has been pointed out in the description of the method that with grasses grown in free nature absolute uniformity could not be expected. Is this irregularity due to the method of sampling? This is not thought to be the case, or regular graphs would not have been obtained at all, and moreover some of the grasses with irregular graphs are from the most protected camps. If it is a climatic factor rain must be considered. It can be seen that with the exception of *Themeda* in Q camp, which was always more moist,

no maximum of phosphorus (sometimes no grass at all) appeared before the heavy September rains in 1926, generally speaking from 8-14 days after the rain. October is rather a dry month and the phosphorus content falls to rise again in the wet November and December. So far rain and phosphorus content could be correlated, but now the difficulty arises. January has very heavy rains, but with the exception of *Eragrostis* a decrease of the phosphorus content is observed; only in February (*Pennisetum*, *Themeda* in O camp) or in March (*Eragrostis*, *Themeda* in Q and H camps) a slight rise occurs again. It seems that at this time of the year no correlation exists between rain, growth of plants and their phosphorus content.

From the investigation of the phosphorus in the grasses of Bechuanaland (1928) it is known that only the early spring rains affect the percentage phosphorus content; wilting early in the season decreases, and first rains increase the phosphorus content. Wilting later in the season as long as it is not permanent or daily has no further effect. On the other hand it has struck the author that in all investigations of the feeding value or mineral content of South African grasses, be they from Bechuanaland, Pretoria or the Eastern Transvaal, minima of nitrogen and mineral content are shown in January.

Regardless of rain the grasses seem to reach a kind of latent stage like in Autumn, the whole process being aggravated by drought. In February or March new growth sets in. What the reason for this phenomenon is, it is impossible to say, but it seems to be inherent in the plant, and not entirely due to an exterior factor.

Thus this period of decrease of the phosphorus in January has to be accepted without explanation. If after January the phosphorus content increases, it is not so much due to the formation of young leaves, which are rather scarce in Ermelo, but to the percentage increase of phosphorus.

In *Hyparrhenia*, a late grass, a good amount of phosphorus is found in the aerial parts from February to April, although the spring value is never again reached. *Themeda* from the same camp also does not decrease to any extent up to May. *Themeda* from Q camp has nearly as high values in March as in spring, but then decreases very quickly. *Pennisetum* (B camp), and *Themeda* (H camp) show a drop in the phosphorus content of their aerial parts as early as March. Unfortunately *Pennisetum* could not be protected after May from grazing animals and the grass was devoured as it came up during the late winter and early spring. Thus the curve from June onwards is no longer a true picture. All the curves indicate that much phosphorus is stored in the subterranean parts of the grasses in autumn and winter and that the total phosphorus in the whole plant does not show as large a percentage decrease as the aerial parts. But nevertheless the total phosphorus continues decreasing when no leaves or haulms are breaking off. It decreases in different proportions for the various grasses. For *Themeda* in H and Q camps it decreases much more than for *Themeda* in O camp. Phosphorus is disappearing, but whence? The only possibility is, to the soil, and as the roots are well developed at this time, it is not likely that the phosphorus returns to the soil through decaying, but through living roots, a

process which has been observed by different authors under very different conditions (Melin, 1914, Hansteen-Cranner, 1922, Ossipowa and Interewa, 1926).

The values during the ensuing spring differ from those during the spring of 1926. For *Hyparrhenia* the spring maximum is noticed three months earlier than during the previous year, thanks to the heavy July rains. It seems striking that this acceleration is not true for the *Themeda* of O and Q camps. But here an external reason hindered the development of green leaves. The grass had not been grazed down for over two years and hence the old leaves of the tufts stood very thickly and thus prevented sprouting until late in the season; only in October did these camps begin to look green when the old leaves were really decaying. With *Eragrostis* the same trouble was encountered as with *Pennisetum*. From the previous July cattle had had access to it and ate all shoots.

At this point the *Themeda* on the firebreak may be discussed. The percentage of phosphorus in it was somewhat lower than that in the specimen on the unburnt veld. The type of curve obtained resembles more that of a grass which had to endure a heavy drought in midsummer, but received rain in spring and in March. The absolute amount of phosphorus in early spring was considerably higher than that of the control, but from November to February it was less. Quite possibly the burnt veld dried out more quickly. The January minimum was lower than that of the control, but the values from February to April were higher on the average. This is the result of burning once every 3 years and under a good rainfall. No figures are available to show how yearly burning affects the total phosphorus content.

The second exceptional case is that of *Cynodon dactylon*, which began to establish itself at the beginning of this investigation. The plants first collected were tiny, but they grew rapidly; from November onwards the rootneck thickened and by January lots of runners had developed, changing the whole appearance of the plant. With these runners the grass is able to carry a tremendous amount of leaves to a small amount of root. Hence the high relative figures for the organs (Table 34). By this time the plant was very well established and in the spring of 1927 the same process of formation of runners again took place, only on a much larger scale. Not only had runners developed during the season, but new leaves were frequently observed, a feature generally so scarce in the Highveld. For these two reasons the graph of the phosphorus content of this grass is most irregular and difficult to interpret. It seems that it was absolutely independent of rain except for the first relative maximum in October. This might be connected with the small transpiration power of *Cynodon dactylon* (Henrici, 1926, p. 701). With many fluctuations the grass reached its highest values during January and April. The fluctuations are enormous and if the graph of this grass were the only one available, one might despair of the method. And yet, in spite of all the fluctuations, the shape of the curve is quite clear, with a maximum in the summer and a minimum in winter 1926 and 1927. Then, in the spring of 1927, came an enormous increase due to increase of plant material and of percentage phosphorus.

Cynodon dactylon is without doubt the plant which produces the most aerial parts per 100 gm. roots if it is once well established, and which has the highest absolute phosphorus content in its aerial parts.

Before going into details of the phosphorus content of the single organs, a comparison may be drawn between the phosphorus content of the aerial parts of a whole tuft of the Ermelo grasses with the Bechuanaland grasses (Tables 52 and 53, Henrici, 1928). With the exception of *Cynodon dactylon* not one of the Highveld grasses approaches the phosphorus content of the Bechuanaland grasses. In Tables 52 and 53 (Henrici, 1928) only the total phosphorus of the whole plant is given, but the value for the aerial parts can be easily calculated by substracting the phosphorus content of the root and rootneck. As has already been pointed out, the values in Bechuanaland vary much more, thus in the following little table the average monthly values are given for comparison.

Phosphorus of aerial parts of a grass corresponding to 100 *gm. roots in gm. Vryburg.*

	1925.				
	Aug.	Sept.	Oct.	Nov.	Dec.
Digitaria eriantha	2·0	1·2	7·1	1·9	1·5
Chrysopogon serrulatus	2·8	1·4	3·8	3·0	3·5

	1926.							
	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.
Digitaria eriantha	2·1	1·6	1·9	2·7	2·2	2·4	2·4	2·8
Chrysopogon serrulatus	3·3	1·6	2·9	1·9	2·6	2·9	2·7	1·2

These few figures characterize the pasture entirely from the point of view of phosphorus content for the years indicated. In the whole table of Bechuanaland grasses there is no figure under 1 while fractions are frequent in the Highveld of Ermelo. From a glance at the figures, it appears that in Bechuanaland the lowest values might be in summer whilst in the Highveld they are in winter. This means that the winter veld in Bechuanaland is a better pasture than the summer veld during a drought. The Highveld round Ermelo is a bad winter pasture, much worse than in the time of the summer minimum of total phosphorus. Whilst 1.5 gm. phosphorus in the aerial parts to 100 gm. roots is high for Ermelo grasses and is, except for *Cynodon*, practically only found in spring, it is considered a low

value for Bechuanaland grasses. When it is taken into consideration how often values under 0.5 gm. occur in Ermelo, it can be seen at what a tremendous disadvantage this veld is against the veld of Bechuanaland. The only compensation for this condition is that the Highveld round Ermelo is better overgrown than Bechuanaland veld. Exact figures are not available for Ermelo, but roughly estimated the tufts stand *twice* as closely as they do in Bechuanaland. where only 16 per cent. of the soil is overgrown by the grass tufts. Even when this is taken into account, the Highveld in Ermelo is still at a great disadvantage.

The result is rather surprising considering the much higher rainfall of the Ermelo district. The rain is apparently reflected only in the high phosphorus percentage in spring. As the absolute phosphorus content of the whole plant is also so much smaller in Ermelo than in Bechuanaland, it might be thought that climatic conditions other than rain influence the intake of phosphorus. But at present it is not possible to make any suggestion.

After this not much has to be added to explain the absolute phosphorus content of the single organs. Only *Themeda* uses the root as a storage organ for phosphorus, all the other grasses store in the rootneck, though the root may double its phosphorus content. At the time of active growth, especially in February, the rootneck is nearly depleted of phosphorus. In some cases (*Pennisetum*, *Monocymbium*, *Harpechloa*, *Eragrostis* and *Cynodon*) this depletion sets in as soon as the haulms increase in size and not at the very first appearance of the haulms. As these are just the grasses which produce seeds, it is likely that the haulms are drawing the phosphorus out of the rootneck. The appearance of haulms is not accompanied by a decrease of the total phosphorus in the green leaves, with the exception of *Pennisetum*. It is striking how small a proportion of the total phosphorus is that contained in the haulms and spikes.

The maximum of phosphorus in the green leaves is due either to a high percentage of phosphorus or to a large amount of leaves. All early spring maxima are due to a high percentage of phosphorus, all late maxima to plenty of green leaves. The appearance of green leaves in spring is not followed by a decrease of the total amount of phosphorus in the rootneck; the phosphorus of the leaves is apparently obtained from the soil. The phosphorus stored in the rootneck disappears only much later. On the other hand, at the time when the phosphorus disappears from the leaves, the rootneck shows a considerable increase. *Re-migration of phosphorus in autumn is beyond doubt, but neither the emptying of the aerial organs nor the storing of the re-migrating phosphorus is quantitative.*

(*c*) The Determination of Some Compounds of Phosphorus.

The first result gathered from Tables 28-39 is that the phosphorus determined in the compounds, does not add up to the total phosphorus, except in a few specific cases. The part not determined is most probably acid soluble phosphorus. If the material is extracted with 0.6 per cent. hydrochloric acid for the determination of phytin, and a determination of the total phosphorus in the extract is made the value not only exceeds that of phosphorus of phytin, but

is generally also much higher than the value for water soluble phosphorus. Especially at times when phytin is nearly absent, is this acid soluble portion of phosphorus considerable. No extractions were made with acid stronger than 0.6 per cent. hydrochloric acid.

The results on the 3 species of grasses are somewhat different and will therefore be discussed separately; after this a comparison will be drawn between the grasses and maize which was grown under the same veld conditions. Even the results for one species are not always the same in different years, depending probably on interior factors of the plant.

Themeda triandra.

Themeda triandra may be discussed first. The leaves in the young green condition contained a good amount of water soluble phosphorus, which later in the season decreased; in the brown leaves in summer it was present in only small amounts; and increased considerably again in autumn and winter. Only a small portion of the water soluble phosphorus can be precipitated with magnesia mixture, that is to say only a small portion of phosphorus is present in the inorganic form, the rest being probably glycerophosphoric acids. In young leaves of the season 1926-27 P_2O_5 originating from phytin was present in much larger amounts than P_2O_5 which could be precipitated with magnesia mixture. In old leaves there was very little, and in brown leaves no P_2O_5 originating from phytin. In the season 1927-28 very little phytin was found, on the other hand more water soluble P_2O_5 and P_2O_5 which could be precipitated with magnesia mixture was present, especially towards the end of summer. P_2O_5 from lecithin was always present only in very small amounts. In young leaves up to 0.018 per cent. P_2O_5 from nucleoproteids was found, whether the leaves appeared in winter or early spring. In old green or brown leaves 0.003-0.006 per cent. P_2O_5 from nucleoproteids was found.

Roots and Rootneck.—The amount of water soluble phosphorus relative to the total phosphorus in root and rootneck was larger than in leaves. Very little if any phytin and very little lecithin was present. The amount of P_2O_5 originating from nucleoproteids varied from 0.003-0.015 per cent., the highest value being at a different time than the highest value in leaves. It may be concluded that young roots are formed and increase of rootneck takes place at another time than the production of young leaves.

Haulms and Spikes.—With the exception of very young spikes and haulms, these organs were very poor in phytin. In the season 1928 no phytin at all was found. In the young haulms a good amount of water soluble phosphorus was found which however decreased rapidly whilst the haulms were ageing. In 1927 no phytin was found in the haulms during October; it only appeared in November in spikes and haulms although the water soluble phosphorus was still dominating; it decreased again as early as December, whilst the water soluble phosphorus was still abundant. In January, 1928, the haulms contained very little phytin and little water soluble phosphorus, the spikes little phytin and less water soluble phosphorus. In February no phytin was found. Haulms and spikes contain at

any time very little lecithin. Except in Spring the phosphorus originating from nucleoproteids was but little, 0.003-0.008 per cent.

Monocymbium cerisaeforme.

The green leaves of *Monocymbium cerisaeforme* contained in 1926 little water soluble phosphorus, but a good amount of non-water soluble phytin. The amount of nucleoproteids was high. The condition improved in February and March, 1927, for the phytin and water soluble phosphorus, but very little phosphorus precipitable with magnesia mixture was present. Strange to say, the phytin did not disappear when the leaves became brown. Phytin was present until July. Young green October leaves contained a lot of water soluble phosphorus and P_2O_5 from the nucleoproteids, but less phytin was present. The phytin decreased rapidly whilst the water soluble phosphorus was still present in large amounts. From January, 1928 little phytin was found; only in February did the water soluble P_2O_5 begin to decrease. More than traces of lecithin were seldom found. The nucleoproteids provided a fairly large amount of phosphorus, often between 0.021-0.036 per cent.; in the old leaves it might only be 0.004 per cent. and in brown leaves between 0.001-0.006 per cent.

The Roots and Rootneck contained a fairly high percentage of water soluble phosphorus, and very little phytin and lecithin. The phosphorus originating from the nucleoproteids, varying from 0.003-0.009 per cent., was once, in March, very high, probably due to the formation of new roots. The proportion of P_2O_5 precipitated by magnesia mixture to water soluble phosphorus fluctuated a good deal, which means that at one time glycero-phosphoric acid, and at another the inorganic phosphorus dominated.

The Spikes and Haulms contained a good amount of water soluble and phytin-phosphorus in March, 1927. Some seeds were produced. Later both the water soluble phosphorus and the phytin decreased. As in the leaves, for a long time the phytin did not disappear entirely from the haulms. Some was still found in July. Later in 1928 no phytin at all was found, but a large amount of water soluble phosphorus was present. No seeds were observed by the time the experiment was discontinued.

Digitaria tricholaenoides.

The Digitaria tricholaenoides taken for these determinations was collected in a camp other than that described in part A of this investigation. The soil was red sand, the same as in O camp and on this soil the grass did exceedingly well and produced plenty of leaves and seeds. The *green leaves* contained plenty of phytin and water soluble phosphorus and a good amount of phosphorus which is precipitated with magnesia mixture during the period of growth. The nucleoproteids were plentiful and even the lecithin was present in higher amounts than in the two other grasses. The brown leaves did not contain phytin. The difference between the brown leaves in winter and summer was very marked, as there was much more water soluble phosphorus in the winter leaves. In young green leaves in April there was no phytin but plenty of water soluble phosphorus and P_2O_5 precipitable with magnesia mixture.

As in B camp, the haulms stood only for a short time.

Haulms and Spikes contained plenty of water soluble phosphorus, P_2O_5 precipitable with magnesia mixture and phytin. The phytin was present in the spikes only as long as there were seeds. As soon as the seeds had fallen, the phytin disappeared. Late haulms were very poor. The content of nucleoproteids was fairly high.

The Root and Rootneck seldom contained phytin and also very little lecithin. Their content of P_2O_5 of the nucleoproteids varied from 0.002-0.009 per cent. The water soluble phosphorus was plentiful, the portion precipitated by magnesia mixture increasing with the advance of the season.

Maize.

Determinations of phosphorus on *maize* were made only at the time of seed formation. In February *the leaves* contained plenty of phytin, more than water soluble phosphorus, the latter being about the same as the phosphorus precipitated by magnesia mixture. The leaves were rich in nucleoproteids. In March when the seeds were ripening all the phosphorus in the leaves was water soluble and the phytin and the phosphorus precipitable with magnesia mixture had decreased.

The Roots and Rootneck in March contained between 40-50 per cent. water soluble phosphorus, a small amount of phytin and nucleoproteids and practically no lecithin. After the seeds had been formed, the water soluble phosphorus decreased, the phytin however, increased, the other fractions being about the same. The nucleoproteids in the roots increased and decreased in the rootneck, because the latter had dried out more and more.

The proceedings in the *haulms* and *spikes* are the most interesting to observe. As the seeds were large enough in maize, they could in the older stages easily be separated from the adhering and enveloping organs, for instance the leaves round the cob and centres.

The *stalks* at the beginning were not poor in phosphorus, containing in February about 50 per cent. water soluble phosphorus—most of it inorganic phosphorus, and not 20 per cent. of phytin phosphorus. At the end of the month, the total phosphorus had decreased by 30 per cent., the proportion of water soluble was higher than before, but the phytin was unchanged. The stalks of fertilized mealies harvested later, behaved similarly only at the end a high amount of insoluble phytin was present. The leaves around the cobs contained a lot of phytin and 60 per cent. of the phosphorus was water soluble at the beginning of February. During the ripening of the grains their total phosphorus content was only about 20 per cent. of the original value, phytin was absent and only about 20 per cent. of the water soluble phosphorus was left. The phosphorus of the sheaths and the centres of the cobs contained was 100 per cent. water soluble.

About 60 per cent. of the phosphorus of *the cobs* in their youngest state was water soluble and 20 per cent. was phytin. Only a small amount of the water soluble phosphorus was inorganic. The total phosphorus still increased, but the phytin showed the highest

increase. This was continued until the grains were ripe; at the end of March 75 per cent. of the phosphorus was water soluble (and 75 per cent. was phytin, the latter being water soluble to a fair extent.) During the whole process the nucleoproteids were abundant. The phosphorus of the ripe grains was made up of 92 per cent. phytin, of which not much was water soluble, and 7 per cent. of nucleoproteid phosphorus. No inorganic phosphorus appeared to be present. Only in the case of the cobs does the phosphorus of the compounds add up to the total phosphorus.

So much for the analytical data. Can any conclusions be drawn as to the different behaviour of these Gramineae? On the whole the roots and rootneck do not show much difference, but with haulms, spikes and leaves, it is quite another matter.

For Monocymbium it may be stated: New haulms were observed twice during the investigation. In March, 1927 leaves were very poor in total phosphorus, and exceedingly poor in phytin and water soluble phosphorus, and no seeds were formed. In December new haulms were formed when the leaves contained more phosphorus, and water soluble phosphorus was plentiful, but little phytin was present. The haulm formation was not followed by a decrease of phosphorus in the leaves. Later in the season some scattered seeds were found.

Themeda showed its first haulms in January, 1927 when leaves were poor in water soluble phosphorus, but not poor in phytin. No seeds were found. In November, 1927 *Themeda* again produced haulms. As in January, no decrease of the phosphorus in the leaves was observed. The leaves contained at this time a good amount of water soluble phosphorus, but little phytin. The latter soon disappeared entirely. No seeds were produced. When the very first determinations were done on *Themeda* with material of the spring of 1926, the leaves contained but little water soluble phosphorus and little phytin and no seeds were produced either.

Digitaria produced haulms only once while it was under observation, in November, 1927. At this time the leaves contained plenty of water soluble phosphorus and plenty of phytin. The leaves decreased in the phosphorus content during the haulm formation. After the seeds had fallen the phytin in the leaves disappeared entirely.

Maize.—A decrease of phosphorus takes place in the leaves, the haulms and the sheaths while the seeds are forming and especially when they are hardening. Plenty of phytin and plenty of water soluble phosphorus are present in the leaves whilst the haulms and cobs are forming. The phytin is not necessarily present in water soluble form.

From these statements it may be concluded that the compounds of phosphorus and not only the *amount* of phosphorus present in the leaves play an important rôle in seed formation. Only when vital seeds are produced do the leaves decrease their phosphorus content during the production, and only when plenty of water soluble phosphorus and a fair amount of phytin is present, are seeds formed. A fair amount of phytin alone or a lot of water soluble phosphorus without phytin cannot be correlated with the formation of vital seeds.

It is by no means the intention of the author to say that the phosphorus, or the phosphorus compounds are the only factors essential to the formation of vital seeds. From manuring experiments (Henrici, 1927) it is known, that the water supply plays a prominent rôle in this process. It is possible that also other factors are correlated.

While an adequate water supply and a fair supply of phosphorus are factors stimulating seed production, it cannot be said that the presence of phytin and plenty of water soluble phosphorus in the leaves, is directly the cause of the seed-production. It might be that the two processes are closely related, that they are both the expression that the plant is vigorous, in good condition. On the other hand *Themeda* also was in quite good condition, and unmanured mealies are not regarded as being in prime condition. And Armoedsvlakte grasses which had no phosphorus precipitable by magnesia mixture, were not always poor, although they did not produce seeds. Naturally, the presence of only water soluble phosphorus and phytin in the leaves cannot be accompanied by seed formation, if the haulms are not pre-formed. Thus *Themeda* several times had a fair amount of water soluble phosphorus or phytin, but no young haulms were present at the time, in November, 1926 and in April, 1927. *Themeda* is therefore not unable to produce these compounds, but it does not produce them at the right time.

DISCUSSION.

In comparing the results of this investigation with the literature, the paper of Brenchley (1929) is of special interest. This author states that the barley plant grows normally if in the first six weeks of its lifetime phosphorus is available to it; if phosphorus is withheld for the first four weeks, no ears are produced. With longer periods of initial deprivation growth was steadily depressed in all respects. This observation may partly give an explanation of the different behaviour of Ermelo and Bechuanaland grasses. In spite of the Ermelo grasses being on the whole poorer than Bechuanaland grasses, they produce more haulms and more vital seeds. Ermelo nearly always has early spring rains, Bechuanaland, however, has generally very late rains, thus the little available phosphorus in the soil is still less available through the drought, which will lead according to Brenchley's result, to poor ears. In this respect Ermelo grasses compare much better.

CONCLUSIONS.

The determination of the phosphorus in the dominating grasses of the Highveld in the Eastern Transvaal reveals the fact that, in spite of the high rainfall these grasses are poor in phosphorus except for early spring. Considering that for the long winter months most of the grasses are without green leaves and the phosphorus content of the brown leaves is exceedingly poor, the pasture is indeed very poor in winter. There are some good grasses in this Highveld pasture, but they are not abundant and if they come up in the spring time, even under very lenient grazing they are eaten and tramped down before they have any chance of producing seeds. The only

advantage the Highveld pasture has over that of dry Bechuanaland is that small amounts of seeds are produced by different grasses, although the seeding is far from being good.

Whilst in the Bechuanaland pasture a minimum of the absolute phosphorus content found during the summer drought in the Highveld pasture, the minimum is always in winter time. The absolute amount of phosphorus (of a plant corresponding to 100 gm. roots) however, is at any time much lower in the Highveld than in Bechuanaland.

There is no need to say that compared with European figures (Godden, 1926) the phosphorus content of the grassveld at Ermelo is very low. Even the average percentage of the P_2O_5 of the non-eaten hill pasture in Europe is about four times higher than the average percentage of the best grasses of the Highveld. Also with Rhodesian autumn figures (Husband, 1929) the Ermelo figures compare unfavourably.

It may be strongly advised not to use the Highveld as winter pasture, or if this is impossible, to avoid certain grey soils of the Highveld as winter pasture. The farmer will recognize this particular grey soil by its flora which is characterized by a great variety of inferior grasses such as *Harpechloa capensis* (called in the Ermelo district Koperdraad) and *Monocymbium cerisaeforme* (Hawergras) and by the absence of *Themeda*, or Rooigras. The soil is so poor that *Digitaria tricholaenoides* (Vingergras) and *Themeda* fail to thrive on it at all.

On the whole the red soil seems to be the best pasture for winter, although the black soil is richer in certain minerals. The richest soil is the black soil but plants wither on the heavy soil much more quickly even when the soil moisture is high than on the light sandy soils as the former does not release water as easily as the sandy soils.

Considering that other plants on the same soil contain more phosphorus the question arises from where these plants get the higher amount of phosphorus. Two sources are possible, either the subsoil, which the grass roots do not reach, is richer in available phosphorus or the phosphorus is present in a form not easily available to grasses. It is known that Gramineae assimilate phosphorite to a much lower degree than, for example, Leguminosae (Kostytschew, 1926, p. 285). Thus both explanations might be possible and only direct experiments could decide.

So much for the agriculturist. From the scientific side it might be emphasized that no doubt remains that autumnal re-migration of P_2O_5 from leaves and haulms takes place. It will be shown in another paper (Henrici, 1929) that the ash content does not increase with the advancing season, so that the figures of P_2O_5 are not disturbed by this factor (Wehmer, 1892). The re-migration of P_2O_5 is, however, not quantitative. About 40 per cent. may remain in the dying leaves; the haulms are emptied to a larger extent. Also the storing of the re-migrating phosphorus in root or rootneck is not quantitative. A portion of it disappears altogether from the plant, and the only possible channel is through the roots as there is no breaking off of leaves at this time of the year. The absolute amount of phosphorus for the whole plant is therefore less in winter than in summer.

Figures are given as to the amount of phosphorus in spikes which is necessary for seed formation. It was observed that only grasses with vital seeds achieved a certain percentage of P_2O_5. The possibility of not only the amount of phosphorus, but also the different compounds of phosphorus in the leaves at the time of haulm formation, influencing the seeding, is discussed. It was observed that only Gramineae which contained a fair amount of phytin and water soluble phosphorus in the leaves showed seed formation; the possibility is, of course, mentioned that other factors may as well play a rôle, and it is by no means denied that the plants producing no seeds are unable to produce phytin or do not contain water soluble phosphorus. It is only stated that they do not contain these compounds at the time of haulm formation.

Whilst some grasses, grown on a soil richer in phosphorus (Henrici, 1928 tables) or manured with phosphorus respond with a considerable increase in their phosphorus, maize on virgin soil and on manured soil in Ermelo showed the same percentage of phosphorus in leaves and spikes. It was only in the quantity of *cobs* that the differently treated plants differed. On the other hand phosphorus manuring without watering did not lead to any increased haulm or seed formation in veld grasses at Ermelo; on the contrary, fewer haulms were formed, though the amount of leaves was increased and the plant proved to be more drought resistant. This latter result induces the belief that besides an adequate phosphorus and water supply another factor, as yet unknown, plays a rôle in the seeding power of the grasses.

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TABLE 2.—*Themeda triandra*. Ermelo O camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
10.8.1926	None	0·06	0·04	—	0·16	0·34	Haulms old.
21.8.1926	None	0·07	0·04	—	0·15	0·22	Haulms old.
4.9.1926	0·38	0·07	0·04	—	0·23	0·27	Haulms old.
16.9.1926	0·38	0·07	None	—	0·20	0·13	—
28.9.1926	0·43	0·06	0·03	—	0·13	0·28	Haulms old.
8.10.1926	0·38	0·08	0·08	—	0·13	0·22	Haulms old.
25.10.1926	0·37	0·08	0·09	—	0·19	0·22	Haulms old.
10.11.1926	0·30	0·10	0·23	0·33	0·27	0·16	New green haulms and spikes.
23.11.1926	0·22	0·11	0·30	0·28	0·14	0·20	Middle aged haulms and spikes.
13.12.1926	0·22	0·06	0·12	0·30	0·07	0·11	Middle aged haulms and spikes.
4.1.1927	0·21	0·08	0·10	0·18	0·15	0·15	Green haulms and green spikes.
18.1.1927	0·21	0·09	0·07	0·12	0·07	0·17	Older haulms.
2.2.1927	0·21	0·05	0·05	0·10	0·08	0·07	Older haulms.
15.2.1927	0·19	0·06	0·03	0·12	0·09	0·13	Shrivelled seeds in spikes.
1.3.1927	0·19	0·07	0·03	0·11	0·14	0·18	Shrivelled seeds in spikes.
15.3.1927	0·25	0·07	0·05	0·07	0·13	0·22	Spikes empty.
7.4.1927	0·28	0·07	0·03	0·06	0·13	0·30	Spikes empty.
26.4.1927	0·25	0·07	0·05	0·11	0·14	0·25	Old haulms.
17.5.1927	0·25	0·08	0·06	—	0·26	0·47	Old haulms.
7.6.1927	0·25	0·12	0·05	—	0·19	0·52	Old haulms.
28.6.1927	None	0·08	None	—	0·16	0·45	—
19.7.1927	0·31	0·13	0·04	—	0·13	—	Old haulms.
9.8.1927	0·53	0·10	0·07	—	0·27	0·37	Old haulms.
26.8.1927	0·51	0·06	0·04	—	0·19	0·29	Old haulms.
15.9.1927	0·63	0·08	0·05	—	0·20	0·35	Old haulms.
29.9.1927	0·63	0·06	0·04	—	0·13	0·29	Old haulms.

TABLE 3.—*Themeda triandra*. Ermelo, H. camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-crown.	Roots.	Remarks.
30.7.1926	No green leaves	0·09	0·05	—	0·18	0·23	Brown old haulms with glumse.
10.8.1926	Do.	0·09	0·04	—	0·23	0·21	Brown old haulms with glumes.
20.8.1926	Do.	0·09	0·04	—	0·19	—	Brown old haulms with glumes.
4.9.1926	Do.	0·09	0·04	—	0·20	0·30	Brown old haulms with glumes.
16.9.1926	Do.	0·09	0·13	—	0·27	0·35	Brown old haulms with glumes.
27.9.1926	0·45	0·07	0·05	—	0·24	0·33	Brown old haulms with glumes.
12.10.1926	0·20	0·08	0·04	—	0·16	0·43	Brown old haulms with glumes.
25.10.1926	0·27	0·06	Brown 0·01 Green 0·18	Green 0·22	0·08	0·09	Young green haulms.
8.11.1926	0·19	0·07	0·14	0·25	0·11	0·11	Young green haulms.
23.11.1926	0·25	0·08	Green 0·32 Brown 0·09	0·23	0·15	0·17	—
13.12.1926	0·20	0·09	0·14	0·24	0·10	0·12	—
4.1.1927	0·19	0·06	0·10	0·19	0·10	0·10	Green spikes with dough seeds.
18.1.1927	0·21	0·07	0·06	0·14	0·13	0·14	Wilted leaves.
1.2.1927	0·22	0·05	0·04	0·08	0·08	0·09	Spikes with shrivelled seeds.
15.2.1927	0·19	0·06	0·04	0·12	0·10	0·13	Shrivelled seeds in spikes.
1.3.1927	0·27	0·08	0·06	0·18	0·15	0·28	New haulms.
15.3.1927	0·16	0·07	0·04	0·08	0·14	0·32	Spikes with few soft seeds.
7.4.1927	0·15	0·07	0·03	0·07	0·12	0·26	Condition of green leaves good, plentiful, haulms old.
26.4.1927	0·22	0·08	0·06	0·08	0·23	0·33	—
17.5.1927	0·14	0·06	0·02	—	0·11	0·17	Spikes found.
7.6.1927	0·12	0·08	None.	—	0·17	0·20	No haulms found.
28.6.1927	0·21	0·08	—	—	0·17	0·31	—
19.7.1927	0·16	0·05	0·03	—	0·13	0·23	Old haulms.
9.8.1927	No green leaves	0·05	0·03	—	0·17	0·38	Old haulms.
25.8.1927	0·46	0·07	0·02	—	0·16	0·41	Old haulms.
15.9.1927	0·61	0·06	0·02	—	0·15	0·31	Old haulms.
30.9.1927	0·50	0·06	0·04	—	0·15	0·28	Old haulms.

TABLE 4.—*Themeda triandra*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
9.8.1926	—	0·05	—	—	0·25	0·32	Haulms brown.
20.8.1926	—	0·08	0·06	—	0·23	0·30	Haulms brown.
7.9.1926	—	0·08	—	—	0·18	0·26	Haulms brown.
17.9.1926	0·40	0·11	0·04	—	0·18	0·23	Haulms brown.
28.9.1926	0·40	0·06	0·05	—	0·18	0·20	Haulms brown.
12.10.1926	0·36	0·10	—	—	0·18	0·25	Haulms brown.
25.10.1926	0·29	0·07	0·09	—	0·10	0·11	Green haulms.
8.11.1926	0·19	0·06	0·14	0·30	0·10	0·09	Green haulms.
18.11.1926	0·18	0·04	0·15	0·33	0·08	0·12	Green haulms with spikes.
23.12.1926	0·21	0·08	0·10	0·24	0·10	0·08	Spikes with dough seeds.

TABLE 4—*continued.*

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root Crown.	Roots.	Remarks.
17.1.1927	0·19	0·09	0·08	0·20	0·13	0·12	Fresh haulms, brown.
31.1.1927	0·19	0·06	0·05	0·14	0·08	0·09	Spikes empty.
14.2.1927	0·23	0·06	0·06	0·09	0·08	0·08	Seeds soft, some shrivelled.
28.2.1927	0·15	0·05	0·05	—	0·10	0·17	Not separated spikes.
14.3.1927	0·17	0·05	0·03	0·09	0·13	0·24	Old spikes and haulms.
6.4.1927	0·15	0·04	0·04	0·08	0·15	0·22	Old spikes and haulms.
25.4.1927	0·21	0·05	0·05	0·08	0·18	0·33	Old spikes and haulms.
16.5.1927	0·12	0·05	0·05	—	0·11	—	Haulms and spikes not separated.
6.6.1927	0·13	0·06	0·04	—	0·17	0·39	Only haulms.
16.6.1927	0·24	0·06	0·04	—	0·17	—	Haulms only.
28.6.1927	None.	Very leached 0·07	0·04	—	—	0·49	Haulms only.
18.7.1927	None.	0·06	0·04	—	0·17	—	Haulms only.
8.8.1927	0·45	0·10	0·09	—	0·27	0·59	Haulms only.
15.8.1927	None.	0·06	None found	—	—	—	—
24.8.1927	None.	0·06	0·04	—	0·22	0·26	Haulms only.
14.9.1927	0·42	0·24	0·08	—	0·07	0·39	Haulms only.
29.9.1927	0·46	0·06	None.	—	0·17	0·26	—

TABLE 5.—*Themeda triandra.* Ermelo, Q camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
9.8.1926	No leaves	0·17	0·04	—	0·25	0·27	Old haulms.
14.8.1926	Do.	0·15	—	—	—	—	—
23.8.1926	Do.	0·13	0·09	—	0·19	0·31	Old haulms, old spikes.
3.9.1926	Do.	0·15	0·06	—	0·19	0·35	Old haulms, old spikes.
16.9.1926	0·62	0·09	0·07	—	0·23	0·37	Leaves fresh, old haulms, old spikes.
27.9.1926	0·43	0·12	0·07	—	0·22	0·33	Do. do.
8.10.1926	0·55	0·09	0·09	—	0·14	0·14	Do. do.
22.10.1926	0·40	0·09	0·07	—	0·16	0·22	Do. do.
11.11.1926	0·35	0·12	0·22	0·33	0·13	0·18	Leaves fresh, new green haulms.
22.11.1926	0·28	0·07	0·16	0·26	0·18	0·20	Leaves fresh, new green haulms, but spikes damaged by rats.
14.12.1926	0·25	0·09	0·11	0·29	0·15	0·16	Leaves fresh.
3.1.1927	0·25	0·07	0·10	0·22	0·11	0·09	Leaves folded, haulms brown. Spikes with dough seeds.
19.1.1927	0·19	0·07	0·06	0·15	0·11	0·09	Dry green leaves, haulms brown. Spikes with shrivelled seeds.
3.2.1927	0·24	0·05	0·04	0·08	0·12	0·07	Green leaves, fresh.
16.2.1927	0·20	0·10	0·04	0·09	0·13	0·19	Green leaves fresh, old spikes, old haulms.
2.3.1927	0·25	0·08	0·03	0·08	0·13	0·21	Green leaves fresh, spikes empty.
16.3.1927	0·17	0·07	0·03	0·09	0·12	0·27	Do. do.
8.4.1927	0·22	0·06	0·03	0·06	0·16	0·31	Do. do.
27.4.1927	0·18	0·10	0·03	0·07	0·17	0·21	Condition of green leaves poor, old haulms.
18.5.1927	0·16	0·06	0·02	0·08	0·17	0·29	Do. do.
8.6.1927	0·54	0·14	0·08	0·10	0·19	0·32	—
28.6.1927	0·29	0·16	0·06	—	0·23	0·32	—
20.7.1927	—	0·10	—	—	—	0·28	No green leaves in the interval.
10.8.1927	No leaves	0·05	0·02	—	0·22	0·25	—
26.8.1927	0·36	0·07	0·03	—	0·25	0·29	—
15.9.1927	0·60	0·07	0·03	—	0·21	0·32	Fresh green leaves.
30.9.1927	0·38	0·05	0·03	—	0·16	0·40	Do. do.

TABLE 6.—*Themeda triandra*. Ermelo, Q camp firebreak. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-Crown.	Roots.	Remarks.
3.9.1926	0·43	—	—	—	0·21	0·17	—
16.9.1926	0·38	0·08	—	—	0·19	0·19	—
27.9.1926	0·38	0·10	—	—	0·14	0·13	—
8.10.1926	0·33	0·09	—	—	0·12	0·10	—
22.10.1926	0·25	0·09	0·20	0·38	0·10	0·22	Green haulms and spikes.
11.11.1926	0·22	0·11	0·16	0·28	0·11	0·22	Do. do.
22.11.1926	0·39	—	0·25	—	0·15	0·26	Haulms not separated from spikes.
14.12.1926	0·23	0·13	0·18	—	0·12	0·14	—
3.1.1927	0·16	0·07	0·09	0·19	0·11	0·09	Green leaves folded.
19.1.1927	0·18	0·08	0·09	0·16	0·13	0·12	—
3.2.1927	0·16	0·05	0·02	0·08	0·07	0·08	Spikes without seeds.
16.2.1927	0·29	0·06	Old haulms 0·10 New haulms 0·15	0·14	0·14	0·34	Old spikes with shrivelled seeds.
2.3.1927	0·22	0·09	0·10	0·13	0·22	0·32	—
16.3.1927	0·24	0·08	0·07	0·09	0·16	0·23	Spikes empty.
8.4.1927	0·28	0·08	0·07	—	0·13	0·36	—
27.4.1927	0·47	0·17	0·26	0·10	0·34	0·63	New haulms.
18.5.1927	0·30	0·13	0·11	—	0·22	0·38	Old brown haulms.
8.6.1927	0·28	0·17	0·06	—	0·18	0·38	Do.
28.6.1927	0·27	0·11	—	—	0·21	0·27	Do.
20.7.1927	No green leaves	0·15	0·05	—	0·23	0·37	Do.
10.8.1927	Do.	0·10	0·06	0·11	0·25	0·32	Do.
26.8.1927	Do.	0·06	0·03	—	0·26	0·47	Do.
15.9.1927	0·72	0·08	0·03	—	0·21	0·36	Do.
30.9.1927	0·52	0·07	0·05	—	0·21	0·29	Do.

TABLE 7.—*Eragrostis* spec. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
9.8.1926	0·25	—	—	—	—	—	Soft, open, green leaves, near water.
6.9.1926	0·49	—	—	—	0·13	0·14	Do. do.
17.9.1926	0·39	0·09	0·04	—	0·20	0·11	Soft, open, green leaves, near water. Old haulms.
28.9.1926	0·38	0·07	0·03	—	0·15	0·08	Do. do.
11.10.1926	0·35	0·08	—	—	0·14	0·08	Soft, open, green leaves, near water.
25.10.1926	0·14	0·06	—	—	0·21	0·10	Green leaves, rolled.
8.11.1926	0·26	0·09	0·13	0·38	0·11	0·10	Green leaves, fresh. New haulms. New spikes.
18.11.1926	0·27	0·07	0·43	0·48	0·11	0·10	Green leaves, fresh. Spikes with seeds.
6.12.1926	0·22	0·07	0·17	0·32	0·16	0·12	Green leaves, fresh. New spikes flowering.

TABLE 7—*continued.*

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
29.12.1926	0·25	0·12	0·16	0·31	0·09	0·09	Green leaves, half folded. Spikes after flowering.
17.1.1927	0·21	0·10	0·08	0·34	0·12	0·08	Leaves fresh. Spikes after flowering.
31.1.1927	0·21	0·11	0·07	0·16	0·09	0·10	Leaves fresh. Old spikes.
14.2.1927	0·21	0·11	—	—	0·11	0·10	Leaves fresh.
28.2.1927	0·33	0·13	0·11	0·14	0·10	0·08	Leaves fresh. Old spikes.
14.3.1927	0·34	0·14	0·05	—	0·15	0·12	Leaves fresh.
6.4.1927	0·32	0·16	0·11	—	0·14	0·10	Leaves fresh. old haulms.
25.4.1927	0·30	0·14	0·09	—	0·17	0·09	Leaves fresh.
16.5.1927	0·26	0·09	0·07	—	0·11	0·13	Do.
6.6.1927	0·28	0·13	0·08	—	0·19	0·14	Do.
28.6.1927	0·16	0·13	0·04	—	0·27	0·12	Old haulms.
18.7.1927	0·23	0·13	—	—	0·16	0·12	New green leaves.
8.8.1927	0·16	0·09	0·03	—	0·21	0·13	Old haulms.
24.8.1927	0·29	0·11	—	—	0·20	0·14	New green leaves.
14.9.1927	0·43	0·06	—	—	0·18	0·11	Do.
29.9.1927	0·38	None.	—	—	—	0·08	Do.

TABLE 8.—*Harpechloa capensis.* Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
12.10.1926	0·14	0·02	0·04	—	0·07	0·13	Leaves fresh. Brown haulms.
25.10.1926	0·18	0·02	0·08	—	0·07	0·06	Do. do.
8.11.1926	0·20	0·03	0·03	—	0·11	0·09	Leaves fresh. Brown haulms with spikes.
18.11.1926	0·12	0·04	0·15	0·24	0·06	—	Leaves rolled. Green haulms and spikes.
1.12.1926	0·21	—	—	—	—	—	Leaves fresh.
29.12.1926	0·15	0·03	0·09	0·24	0·05	0·05	Green haulms. Spikes before flowering.
17.1.1927	0·14	0·04	0·07	0·24	0·06	0·03	Green Haulms. II Flowering spikes.
31.1.1927	0·13	0·04	0·07	0·14	0·07	0·05	New haulms. II Still flowering.
14.2.1927	0·21	0·05	0·02	0·14	0·07	0·05	Old haulms. Spikes flowering, but decidedly older.
28.2.1927	0·14	0·04	0·09	0·09	0·09	0·08	Middle-aged haulms. Spikes empty.
14.3.1927	0·11	0·02	0·02	0·30	0·05	0·05	Old haulms. Younger spikes.
6.4.1927	0·16	0·03	0·04	0·15	0·06	0·06	Old haulms. Spikes mostly empty.
25.4.1927	0·10	0·03	0·02	0·05	0·06	0·07	Old haulms. Old spikes.
16.5.1927	0·09	0·03	0·02	0·03	0·08	0·12	Old haulms. Old spikes.
6.6.1927	0·10	0·04 { Old 0·03 White 0·01		—	0·09	0·16	{ Old white haulms. Old haulms.
28.6.1927	0·15	0·04	0·02	—	0·12	0·14	Old haulms.
18.7.1927	—	0·04	0·03	—	0·11	0·09	Do.
8.8.1927	0·25	0·04	0·02	—	0·11	0·11	Do.
24.8.1927	0·27	0·04	0·03	—	0·15	0·12	Do.
14.9.1927	0·27	0·03	0·02	—	0·08	0·09	Do.
29.9.1927	0·37	0·03	0·02	—	0·11	0·12	Do.

TABLE 9.—*Monocymbium cerisaeforme*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
23.7.1926	—	0·05	0·04	—	0·25	0·18	Old haulms.
9.8.1926	—	0·05	0·02	—	0·19	0·23	Do.
20.8.1926	—	0·06	0·03	—	0·17	0·23	Do.
7.9.1926	—	0·04	0·03	—	0·21	0·30	Do.
17.9.1926	0·36	0·07	0·06	—	0·20	0·23	Do.
28.9.1926	0·48	0·06	0·02	—	0·17	0·19	Do.
11.10.1926	0·41	0·05	0·04	—	0·17	0·16	Do.
25.10.1926	0·23	0·03	0·07	—	0·14	0·08	Do.
8.11.1926	0·25	0·05	0·03	—	0·11	0·11	Do.
18.11.1926	0·26	0·05	0·03	—	0·12	0·11	Old haulms, mostly broken down since last sampling.
13.12.1926	0·24	—	None.	—	0·10	0·10	—
17.1.1927	0·15	0·04	0·12	—	0·08	0·08	New haulms.
31.1.1927	0·14	0·04	0·11	—	—	0·07	Do.
14.2.1927	0·15	0·04	0·12	0·21	0·13	0·11	Spikes flowering.
28.2.1927	0·14	0·06	0·07	0·21	0·08	0·07	New haulms.
14.3.1927	0·12	0·04	0·07	0·17	0·13	0·14	Spikes mostly empty.
16.4.1927	0·17	0·07	0·03	0·15	0·15	0·15	Haulms old.
25.4.1927	0·13	0·10	0·04	0·11	0·13	0·14	Most spikelets empty, old haulms.
16.5.1927	0·32	0·09	0·07	0·07	0·22	0·21	Spikelets fallen, spathes.
6.6.1927	None.	0·12	0·07	0·10	0·20	0·25	Haulms with spathes.
28.6.1927	None.	0·10	0·05	—	0·14	0·15	Old haulms.
18.7.1927	None.	0·06	0·03	—	0·15	0·21	Do.
8.8.1927	None.	0·06	0·07	—	0·14	0·19	Do.
24.8.1927	None.	0·06	0·05	—	0·25	0·25	Do.
14.9.1927	0·43	0·08	0·05	—	0·19	0·22	Do.
29.9.1927	0·58	0·05	0·04	—	0·16	0·20	Do.

TABLE 10.—*Digitaria tricholaenoides*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Remarks.
11.10.1926	0·23	0·05	—	—	0·11	0·04	—
25.10.1926	0·23	0·04	—	—	0·11	0·04	—
8.11.1926	0·20	0·07	—	—	0·09	0·09	—
18.11.1926	0·16	0·04	—	—	0·11	0·11	—
13.12.1926	0·16	0·06	0·13	0·23	0·09	0·08	Green haulms. Spikes before flowering.
29.12.1926	0·18	0·06	0·12	0·21	0·08	0·07	—
21.1.1927	0·14	0·08	—	—	0·07	0·07	—
31.1.1927	0·15	0·05	—	—	0·08	0·07	—
14.2.1927	0·26	0·08	—	—	0·07	0·10	—
28.2.1927	0·30	0·09	—	—	0·08	0·08	—
14.3.1927	0·17	0·12	—	—	0·07	0·05	—
6.4.1927	0·22	0·09	—	—	0·12	0·13	—
25.4.1927	0·15	0·09	—	—	0·12	0·17	—
16.6.1927	0·12	0·07	—	—	0·14	0·12	—
6.6.1927	None.	0·07	—	—	0·15	0·17	—
28.6.1927	None.	0·07	—	—	0·12	0·27	—
18.7.1927	None.	0·07	—	—	0·12	0·13	—
8.8.1927	None.	0·06	—	—	0·12	0·16	—
24.8.1927	None.	0·07	—	—	0·10	0·11	—
14.9.1927	0·42	0·04	—	—	0·12	0·19	—
29.9.1927	0·43	0·05	—	—	0·12	0·15	—

TABLE 11.—*Pennisetum Thunbergii*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Rootneck.	Roots.	Remarks.
6.9.1926	0·46	—	—	—	—	—	—
17.9.1926	0·50	0·07	—	—	0·30	0·17	—
28.9.1926	0·54	0·05	—	—	0·29	0·19	—
11.10.1926	0·30	0·09	—	—	0·25	0·13	—
25.10.1926	0·30	0·13	—	—	0·17	0·12	—
8.11.1926	0·30	0·07	—	—	0·27	0·15	—
18.11.1926	0·28	0·08	—	—	0·17	0·12	—
6.12.1926	0·25	0·10	0·24	0·43	0·17	0·10	Flowering spikes. Green haulms.
29.12.1926	0·31	0·12	0·24	0·43	0·16	0·12	Spikes with dough seeds.
17.1.1927	0·21	0·07	0·13	0·39	0·15	0·09	Spikes with a few hard seeds, but mainly unripe seeds.
31.1.1927	0·18	0·06	0·07	0·29	0·11	0·10	Seeds falling.
14.2.1927	0·27	0·07	0·12	—	0·13	0·10	—
28.2.1927	0·19	0·07	Old 0·04 / Younger 0·16	—	0·27	0·12	—
14.3.1927	0·25	0·11	—	—	0·29	0·14	—
6.4.1927	0·25	0·09	0·18	—	0·28	0·12	—
25.4.1927	0·24	0·09	—	—	0·25	0·17	—
16.5.1927	0·20	0·09	0·08	—	0·27	0·14	—
6.6.1927	0·30	0·11	—	—	0·37	0·17	—
28.6.1927	0·40	0·12	—	—	0·29	0·17	—
18.7.1927	0·25	0·13	—	—	0·29	0·19	—
8.8.1927	—	0·07	—	—	0·29	0·18	—
24.8.1927	0·75	0·12	—	—	0·27	0·19	—
14.9.1927	0·81	0·13	—	—	0·34	0·17	—
29.9.1927	0·61	0·09	—	—	0·29	0·17	—

TABLE 12.—*Arundinella Ecklonii*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Rootcrown.	Roots.	Remarks.
23.7.1926	—	0·06	0·06	—	—	—	Dry haulms with glumes.
11.10.1926	0·33	0·06	0·06	—	0·08	0·13	Dry haulms.
25.10.1926	0·21	0·03	0·06	—	0·11	0·14	Dry haulms.
3.11.1926	0·23	0·06	0·04	—	0·14	0·09	Dry haulms.
18.11.1926	0·23	0·05	0·04	—	0·12	0·08	Dry haulms.
29.12.1926	0·18	0·05	Old 0·02 Green 0·10	—	0·14	0·08	Dry and green haulms.
31.1.1927	0·17	0·04	Old 0·03 Green 0·10	0·14	0·08	0·06	Dry and green haulms with spikes.
14.2.1927	0·22	0·04	Old 0·03 Green 0·11	—	0·11	0·10	Green haulms, not young.
25.3.1927	0·12	0·04	Old 0·05 Green 0·09	0·09	0·13	0·06	—
6.6.1927	0·30	0·06	New 0·06 Old 0·02	—	0·28	0·17	—

TABLE 13.—*Setaria imberbis*. Ermelo, B camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Rootcrown.	Roots.	Remarks.
23.7.1926	0·18	0·07	0·04	—	0·07	0·16	Haulms with empty spikes.
11.10.1926	0·23	0·07	0·07	—	0·23	0·13	Rolled leaves.
26.10.1926	0·23	0·05	0·08	—	0·20	0·09	—
3.11.1926	0·22	0·05	0·03	—	0·14	0·12	—
18.11.1926	0·23	0·04	None.	—	0·13	0·10	Open leaves.
29.12.1926	0·18	0·04	Leached 0·03 Yellow fresh 0·07	Fresh 0·12	0·10	0·12	Rolled leaves.
31.1.1927	0·14	0·04	Too little material.	—	0·07	0·09	—

TABLE 14.—*Hyparrhenia Buchanani*. Ermelo, O camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Rootneck.	Roots.	Remarks.
26.7.1926	0·16	0·10	0·06	—	0·18	0·17	Dry haulms with empty spikes.
20.8.1926	0·38	0·09	0·06	—	0·12	0·13	Dry haulms with empty spikes.
3.9.1926	0·33	0·08	0·06	—	0·12	0·14	Dry haulms with empty spikes.
16.9.1926	0·46	0·08	0·04	—	0·15	0·13	Dry haulms with empty spikes.
27.9.1926	0·42	0·09	0·06	—	0·12	0·12	Dry haulms with empty spikes.
13.10.1926	0·29	0·06	0·04	—	0·08	0·09	Dry haulms with empty spikes.
25.10.1926	0·23	0·07	0·03	0·06	0·10	0·09	Dry haulms with old spikes.
10.11.1926	0·25	0·06	0·04	0·07	0·13	0·12	Dry haulms with old spikes.
23.11.1926	0·25	0·07	0·04	—	0·13	0·10	Dry haulms with old spikes.
13.12.1926	0·22	0·11	0·08	—	0·16	0·15	Dry haulms with old spikes.
4.1.1927	0·19	0·08	0·04	—	0·10	0·09	Dry haulms with old spikes.
18.1.1927	0·18	0·07	0·04	—	0·12	0·10	Dry haulms with old spikes.
1.2.1927	0·14	0·04	New 0·08 Old 0·03	—	0·08	0·07	New and old haulms.
15.2.1927	0·21	0·08	New 0·09 New 0·03	0·26	0·11	0·09	New spikes, seedforming, small, soft.
1.3.1927	0·17	0·08	New 0·08 Old 0·03	0·24	0·15	0·07	New haulms and spikes.
15.3.1927	0·35	0·09	Older 0·12 Young 0·18	0·38	0·15	0·04	This year's older and young haulms with Spikes.
7.4.1927	0·29	0·09	Old 0·03 New 0·13	0·35	0·13	0·11	Spikes about half with ripe seeds.
26.4.1927	0·27	0·08	New 0·07 Leached 0·02	0·26	0·14	0·13	Seeds falling ripe. Old leaded and younger haulms.
17.5.1927	0·30	0·06	Young 0·07 Old 0·03	0·38	0·17	0·14	Spikes with immature seeds, mature seeds and fallen seeds.
7.6.1927	0·33	0·10	Old 0·14 Older 0·04	—	0·16	0·14	—
28.6.1927	0·31	0·11	Younger 0·13 Old 0·07	—	0·16	0·14	—
19.7.1927	0·35	0·12	Younger 0·14 Old white 0·04	—	0·22	0·13	—
9.8.1927	0·37	0·10	0·07	—	0·22	0·17	—
26.8.1927	0·51	0·12	0·14	—	0·22	0·16	—
15.9.1927	0·49	0·09	0·05	—	0·14	0·13	—
30.9.1927	0·60	0·13	0·12	—	0·25	0·16	—

TABLE 15:—*Cynodon Dactylon*. Ermelo, Q camp. Phosphorus content as percentage phosphoric oxide on plant dry matter throughout the year.

Date.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	State of Haulms.	Roots.	Rootcrown.	Remarks.
28.7.1926	0·27	—	—	—	—	0·17	—	Young green leaves.
9.8.1926	0·25	—	—	—	—	0·22	0·25	1–2 c.m. long, leaves open.
20.8.1926	0·31	—	—	—	—	0·21	0·33	—
3.9.1926	0·50	—	—	—	—	0·17	0·37	—
16.9.1926	0·53	—	—	—	—	0·17	0·43	—
27.9.1926	0·36	—	—	—	—	0·11	0·27	—
8.10.1926	0·38	—	—	—	—	0·22	0·20	—
22.10.1926	0·31	—	—	—	—	0·14	0·25	—
11.11.1926	0·27	0·2	0·26	—	Young halms and spikes not separated	0·11	0·17	Old leaves of this year.
22.11.1926	0·28	—	0·30	—	,,	0·14	0·15	—
14.12.1926	0·29	—	0·33	—	Spikes flowering	0·12	0·14	—
3.1.1927	0·27	—	0·25	0·37	,,	0·16	Runner 0·14 R. Cr. 0·17	Leaves fresh.
19.1.1927	0·24	0·10	0·18	—	Haulms green	0·12	Runner 0·17 R. Cr. 0·14	Leaves fresh.
3.2.1927	0·17	0·03	0·14	—	Spikes and haulms old	0·07	Runner 0·09 R. Cr. 0·08	—
16.2.1927	0·27	0·12	0·11	—	Spikes and haulms old	0·10	Runner 0·15 R. Cr. 0·14	—
2.3.1927	0·34	0·16	0·18	—	Haulms green	0·18	R. Cr. 0·15	—
16.3.1927	0·22	0·11	(*a*) 0·16 (*b*) 0·12	—	(*a*) Green haulms (*b*) Old brown haulms	0·12	Runner 0·15 R. Cr. 0·14	—
8.4.1927	0·31	0·11	0·12	—	Old brown haulms	0·12	Runner 0·21 R. Cr. 0·15	Fresh.
27.4.1927	0·33	0·08	0·19	—	Haulms and spikes	0·12	Runner 0·19 R. Cr. 0·15	Leaves largely nipped by frost.
18.5.1927	0·51	0·15	0·22	—	Haulms	0·19	Runner 0·23 R. Cr. 0·19	After rain.
8.6.1927	0·34	0·16	0·18	—	Haulms and spikes	0·14	Runner 0·24 R. Cr. 0·15	—
28.6.1927	0·30	0·11	0·17	—	Haulms	0·14	Runner 0·18 R. Cr. 0·16	—
26.7.1927	None.	0·11	0·18	—	Haulms	0·13	Runner 0·14 R. Cr. 0·17	—
10.8.1927	None.	0·12	0·24	—	Old Haulms	0·16	R. Cr. 0·26	—
26.8.1927	No leaves.	0·11	0·39	—	Old Haulms	0·19	R. Cr. 0·49	—
15.9.1927	0·83	0·10	0·36	—	Haulms	0·20	Runner 0·46 R. Cr. 0·45	Very small leaves.
30.9.1927	0·95	0·11	0·42	—	Haulms	0·20	Runner 0·53 R. Cr. 0·44	Very small leaves.

TABLE 20.—*Themeda triandra*. Ermelo, O camp.
Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root=1.					Amount of P_2O_5 in the Different Organs.						Total.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
10.8.26	—	6·2	2·2	—	3·1	0·34	—	0·37	0·09	—	0·50	1·30	0·46
21.8.26	—	7·2	2·6	—	4·2	0·22	—	0·50	0·10	—	0·63	1·45	0·60
6.9.26	0·3	5·0	1·0	—	3·0	0·27	0·13	0·35	0·04	—	0·69	1·48	0·52
16.9.26	0·2	3·2	0·6	—	0·7	0·13	0·08	0·22	—	—	0·14	0·57	0·30
28.9.26	1·6	15·3	1·0	—	7·0	0·28	0·72	0·92	0·03	—	0·91	2·86	1·67
8.10.26	1·2	3·7	1·4	—	2·6	0·22	0·46	0·30	0·11	—	0·34	1·43	0·87
25.10.26	1·8	5·4	1·2	—	1·6	0·22	0·66	0·43	0·11	—	0·30	1·72	1·20
11.11.26	13·0	6·5	4·2	1·8	2·2	0·16	0·90	0·65	0·99	0·57	0·60	†3·87	†3·11
23.11.26	4·6	7·6	2·6	0·4	4·0	0·20	1·02	0·84	0·78	0·11	0·56	3·51	2·75
13.12.26	5·1	7·2	4·7	0·3	3·2	0·11	1·12	0·43	0·56	0·10	0·22	2·54	2·21
4.1.27	2·8	1·0	2·1	0·5	4·5	0·15	0·58	0·08	0·21	0·09	0·68	1·79	0·96
18.1.27	2·5	4·5	2·1	0·5	3·7	0·17	0·53	0·41	0·15	0·06	0·26	1·58	1·15
1.2.27	3·7	4·3	2·2	0·5	3·0	0·07	0·77	0·21	0·11	0·05	0·24	1·46	1·15
15.2.27	4·6	4·4	2·1	0·3	3·7	0·13	0·89	0·26	0·06	0·03	0·33	1·70	1·24
1.3.27	2·6	4·2	1·3	—	2·8	0·18	0·50	0·29	0·04	—	0·40	1·41	0·83
15.3.27	3·4	4·6	1·4	0·2	2·8	0·22	0·84	0·32	0·07	—	0·35	1·80	1·23
7.4.27	1·8	6·9	2·6	0·2	3·6	0·30	0·51	0·48	0·08	0·01	0·47	1·85	1·08
26.4.27	2·8	5·1	1·3	0·2	3·2	0·25	0·69	0·42	0·07	0·02	0·45	1·90	1·20
17.5.27	1·1	8·0	2·9	—	3·3	0·47	0·28	0·64	0·17	—	0·86	2·42	1·09
7.6.27	0·2	5·1	0·5	—	2·9	0·52	0·05	0·61	0·03	—	0·55	1·76	0·69
28.6.27	—	7·3	0·2	—	3·5	0·45	—	0·56	—	—	0·56	1·57	0·56
19.7.27	0·3	7·0	0·3	—	4·8	0·40	0·10	0·91	0·01	—	0·62	2·04	1·02
9.8.27	0·06	4·4	0·3	—	2·4	0·37	0·03	0·44	0·02	—	0·65	1·51	0·49
26.8.27	0·1	6·2	1·5	—	2·6	0·29	0·05	0·37	0·06	—	0·49	1·26	0·48
15.9.27	0·4	7·3	0·8	—	3·3	0·35	0·13	0·58	0·04	—	0·66	1·76	0·75
29.9.27	0·7	4·1	0·9	—	2·2	0·29	0·46	0·24	0·04	—	0·29	1·32	0·74

† Absolute maximum.

TABLE 21.—*Themeda triandra*. Ermelo, B camp.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
20.8.1926	—	2·7	0·6	—	1·6	0·30	—	0·21	0·04	—	0·29	0·84	0·25
7.9.1926	0·3	1·6	—	—	2·2	0·26	0·12	0·11	—	—	0·40	0·89	0·23
17.9.1926	0·2	2·8	1·3	—	1·9	0·23	0·08	0·31	0·05	—	0·34	1·01	0·44
28.9.1926	1·0	4·7	0·7	—	2·3	0·20	0·40	0·52	0·03	—	0·41	1·56	0·95
12.10.1926	1·2	5·0	3·0	—	1·8	0·25	0·43	0·50	0·15	—	0·33	1·66	1·08
25.10.1926	1·6	1·9	1·5	—	2·7	0·11	0·46	0·16	0·13		0·27	1·13	0·75
8.11.1926	3·2	5·8	2·0	0·8	2·8	0·09	0·60	0·35	0·28	0·25	0·28	1·85	1·48
18.11.1926	0·9	0·7	0·15	0·12	1·5	0·12	0·17	0·03	0·02	0·04	0·12	0·50	0·26
24.12.1926	2·1	2·0	1·2	0·3	2·3	0·08	0·43	0·16	0·12	—	0·23	1·02	0·71
17.1.1927	2·1	1·2	1·1	0·3	2·6	0·12	0·40	0·08	0·09	0·06	0·34	1·09	0·53
31.1.1927	2·1	2·4	1·9	0·3	3·3	0·09	0·40	0·14	0·10	0·04	0·26	1·03	0·68
14.2.1927	1·9	3·6	1·6	0·2	2·0	0·08	0·44	0·21	0·09	0·02	0·16	1·00	0·76
28.2.1927	2·5	2·0	2·2	0·5	1·3	0·17	0·37	0·10	0·11	0·05	0·13	0·93	0·63
14.3.1927	2·4	3·5	0·9	0·1	2·6	0·24	0·41	0·18	0·03	0·01	0·34	1·21	0·63
6.4.1927	2·8	4·1	2·8	0·2	2·7	0·22	0·43	0·16	0·11	0·01	0·40	1·33	0·71
25.4.1927	1·8	3·6	1·1	0·1	2·0	0·33	0·39	0·18	0·06	0·01	0·36	1·33	0·64
6.6.1927	0·25	2·6	0·5	—	1·6	0·39	0·03	0·16	0·02	—	0·27	0·87	0·21
28.6.1927	—	4·8	0·9	—	2·3	0·49	—	0·29	0·04	—	0·39	1·21	0·33
18.7.1927	—	3·1	1·0	—	1·9	0·49	—	0·19	0·04	—	0·32	1·04	0·23
8.8.1927	0·15	6·1	1·6	—	2·6	0·59	0·07	0·61	0·14	—	0·69	2·10	0·82
24.8.1927	—	2·5	0·7	—	1·5	0·26	—	0·15	0·03	—	0·33	0·77	0·18
14.9.1927	0·5	2·1	0·2	—	1·2	0·39	0·21	0·50	0·01	—	0·08	1·19	0·72
29.9.1927	0·5	1·1	—	—	1·4	0·26	0·23	0·07	—	—	0·24	0·80	0·30

TABLE 22.—*Themeda triandra*. Ermelo, H camp.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
10.8.1926	—	2·6	1·1	—	0·9	0·21	—	0·24	0·04	—	0·22	0·71	0·28
20.8.1926	—	4·0	1·0	—	1·2	0·21	—	0·36	0·04	—	0·23	0·84	0·40
4.9.1926	—	6·0	1·4	—	1·8	0·30	—	0·54	0·04	—	0·36	1·24	0·58
16.9.1926	0·13	2·6	0·5	—	0·6	0·35	0·06	0·23	0·06	—	0·16	0·86	0·35
27.9.1926	0·4	6·4	1·3	—	1·1	0·33	0·18	0·45	0·06	—	0·26	1·28	0·69
12.10.1926	0·4	4·0	1·4	—	2·0	0·43	0·08	0·32	0·06	—	0·32	1·21	0·46
25.10.1926	2·0	5·0	Green 0·18; Brown 0·5	Green 0·15	2·3	0·09	0·54	0·30	0·09	0·03	0·18	1·23	0·96
8.11.1926	2·7	4·4	2·2	1·7	3·2	0·11	0·51	0·31	0·31	0·43	0·35	2·02	1·56
23.11.1926	4·1	4·1	Old 0·5; Green 1·0	0·2	1·6	0·17	1·03	0·33	Old 0·05; Green 0·30	0·05	0·24	2·17	1·76
13.12.1926	2·9	1·9	1·0	0·25	3·2	0·12	0·58	0·14	0·14	0·06	0·32	1·36	0·92
4.1.1927	3·7	1·6	3·3	0·6	3·2	0·10	0·70	0·10	0·33	0·10	0·32	1·65	1·23
18.1.1927	3·2	2·0	1·4	0·75	3·0	0·14	0·67	0·14	0·09	0·11	0·39	1·54	1·01
1.2.1927	2·0	0·8	0·9	0·25	2·3	0·09	0·44	0·04	0·04	0·02	0·18	0·81	0·54
15.2.1927	2·6	1·1	0·8	0·14	1·5	0·13	0·50	0·07	0·03	0·02	0·15	0·90	0·62
1.3.1927	3·1	2·7	1·4	0·6	2·1	0·28	0·83	0·21	0·08	0·11	0·31	1·82	1·23
15.3.1927	2·7	2·5	1·6	0·2	2·7	0·32	0·43	0·18	0·06	0·02	0·38	1·39	0·69
7.4.1927	3·7	2·0	1·1	0·3	2·9	0·26	0·56	0·14	0·03	0·02	0·35	1·36	0·75
26.4.1927	2·1	3·9	1·9	0·1	2·5	0·33	0·46	0·31	0·11	0·01	0·48	1·70	0·89
17.5.1927	0·6	2·1	0·2	—	2·0	0·17	0·08	0·13	—	—	0·22	0·60	0·21
7.6.1927	1·0	3·1	—	—	4·2	0·31	0·12	0·23	—	—	0·71	1·39	0·37
28.6.1927	0·4	5·3	0·2	—	2·7	0·31	0·05	0·42	—	—	0·46	1·24	0·47
19.7.1927	—	3·1	—	—	1·9	0·23	—	0·15	0·03	—	0·25	0·66	0·18
9.8.1927	—	2·1	0·33	—	1·7	0·38	—	0·10	0·01	—	0·29	0·78	0·11
25.8.1927	0·8	3·9	0·6	—	2·2	0·41	0·37	0·28	0·01	—	0·35	1·42	0·66
15.9.1927	0·6	5·6	2·8	—	2·6	0·31	0·37	0·34	0·06	—	0·39	1·47	0·77
30.9.1927	0·7	3·7	1·2	—	2·3	0·28	0·35	0·22	0·05	—	0·35	1·25	0·62

TABLE 23.—*Themeda triandra*. Ermelo, Q camp.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
9.8.1926	—	7·2	5·0	—	3·4	0·27	—	1·22	0·20	—	0·85	2·54	1·42
23.8.1926	—	4·7	4·3	—	1·5	0·31	—	0·61	0·39	—	0·28	1·59	1·00
4.9.1926	—	2·2	1·7	—	1·3	9·35	—	0·33	0·10	—	0·25	1·02	0·43
16.9.1926	0·22	1·7	0·2	—	2·3	0·37	0·14	0·15	0·15	0·01	0·52	1·34	0·45
28.9.1926	0·13	1·5	1·1	—	1·4	0·33	0·05	0·20	0·08	—	0·31	0·97	0·64
8.10.1926	3·0	1·7	—	—	3·7	0·14	1·65	0·15	—	—	0·52	2·46	1·80
23.10.1926	0·75	4·25	0·6	—	1·5	0·22	0·30	0·38	0·04	—	0·24	1·18	0·72
14.12.1926	3·0	2·9	2·5	0·6	1·3	0·16	0·75	0·26	0·28	0·17	0·20	1·82	1·46
3.1.1927	2·8	3·5	2·5	0·6	2·1	0·09	0·70	0·25	0·25	0·13	0·23	1·65	1·32
19.1.1927	2·2	4·1	3·1	0·8	3·8	0·09	0·42	0·29	0·19	0·12	0·42	1·53	1·02
3.2.1927	2·0	1·0	4·6	0·15	1·5	0·07	0·48	0·05	0·18	—	0·18	0·96	0·71
16.2.1927	2·3	1·0	0·9	0·13	1·6	0·19	0·46	0·10	0·04	0·01	0·21	1·01	0·61
2.3.1927	2·7	2·5	1·3	0·3	2·3	0·21	0·65	0·12	0·05	0·03	0·28	1·34	0·85
16.3.1927	5·5	4·5	2·3	0·4	3·7	0·19	1·10	0·45	0·09	0·04	0·48	2·36	1·69
8.4.1927	4·1	2·1	1·4	1·17	3·7	0·31	0·90	0·13	0·04	0·01	0·59	1·98	1·08
27.4.1927	1·3	1·4	0·8	0·13	1·4	0·21	0·24	0·14	0·02	0·01	0·24	0·86	0·41
18.5.1927	0·8	2·3	1·2	—	1·7	0·21	0·12	0·14	—	0·03	0·30	0·80	0·29
8.6.1927	0·4	2·0	0·6	0·04	1·5	0·32	0·19	0·28	—	0·04	0·29	1·12	0·51
28.6.1927	0·09	2·3	1·4	—	1·6	0·32	0·02	0·37	—	0·02	0·37	1·10	0·42
20.7.1927	—	1·7	—	—	1·6	0·28	—	0·17	—	—	0·35	0·80	0·17
10.8.1927	—	2·0	0·4	—	1·4	0·25	—	0·10	—	0·01	0·30	0·66	0·11
26.8.1927	0·15	3·5	0·5	—	1·7	0·39	0·03	0·25	—	0·15	0·43	1·25	0·43
15.9.1927	0·40	3·7	2·4	—	2·2	0·40	0·24	0·26	—	0·06	0·46	1·42	0·56
30.9.1927	1·33	3·5	0·6	—	1·6	0·40	0·51	0·18	—	0·18	0·25	1·52	0·87

TABLE 24.—*Themeda triandra*. Ermelo, Q camp. Firebreak.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
4.9.1926	0·5	—	—	—	7·9	0·17	0·21	—	—	—	1·07	1·45	0·21
16.9.1926	0·9	3·1	—	—	10·0	0·19	0·34	0·25	—	—	1·90	2·68	0·53
27.9.1926	1·2	4·3	—	—	3·2	0·13	0·46	0·43	—	—	0·45	1·47	0·46
8.10.1926	6·4	5·8	—	—	3·3	0·10	2·11	0·53	—	—	0·40	3·14	2·64
22.10.1926	4·0	1·1	0·2	0·6	3·2	0·26	1·56	0·12	0·15	—	0·48	3·57	2·83
22.11.1926	1·2	—	0·85	—	1·2	0·26	0·47	—	0·22	—	0·18	1·13	0·69
14.12.1926	2·9	0·25	2·1	0·5	3·4	0·14	0·66	0·03	0·27	—	0·41	1·51	0·96
3.1.1927	2·8	3·5	2·5	0·6	2·1	0·09	0·69	0·25	0·23	0·11	0·23	1·60	1·28
19.1.1927	1·7	0·4	0·3	0·1	2·8	0·12	0·30	0·03	0·03	0·02	0·36	0·86	0·38
3.2.1927	1·3	1·1	0·6	0·15	2·6	0·08	0·21	0·06	0·01	0·01	0·18	0·55	0·29
16.2.1927	1·7	1·05	1·1	0·4	2·6	0·34	0·50	0·06	0·06	0·17	0·60	1·73	0·79
2.3.1927	6·7	2·3	2·3	0·5	5·3	0·32	1·48	0·20	0·23	0·06	1·17	3·46	1·97
16.3.1927	3·4	1·1	0·3	0·18	2·8	0·23	0·83	0·09	0·02	0·02	0·45	1·64	0·96
8.4.1927	7·3	1·0	2·1	—	1·9	0·36	2·04	0·08	0·15	—	0·25	2·88	2·17
27.4.1927	3·1	1·5	0·9	0·08	1·5	0·65	0·25	0·01	0·51	0·23	1·45	3·06	1·00
18.5.1927	0·8	3·8	0·7	—	3·6	0·38	0·24	0·49	0·08	—	0·80	1·99	0·81
8.6.1927	0·09	3·4	0·4	—	1·5	0·38	0·03	0·58	0·02	—	0·27	1·28	0·63
28.6.1927	0·3	3·6	—	—	2·2	0·27	0·08	0·40	—	—	0·46	1·21	0·48
20.7.1927	—	1·7	0·12	—	2·1	0·37	—	0·26	0·01	—	0·46	1·10	0·27
10.8.1927	—	2·1	1·2	0·13	2·1	0·32	—	0·21	0·13	0·01	0·53	1·20	0·35
26.8.1927	—	2·4	3·1	—	1·75	0·47	—	0·06	0·03	—	0·21	1·07	0·23
15.9.1927	0·25	2·8	0·14	—	0·9	0·29	0·18	0·20	0·07	—	0·19	0·93	0·45
30.9.1927	0·9	2·1	0·6	—	1·75	0·29	0·47	0·15	0·03	—	0·37	1·31	0·65

TABLE 25.—*Monocymbium Cerisaeforme*. Ermelo, B camp.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total P_2O_5.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
9.8.1926.....	—	2·8	4·3	—	1·0	0·23	—	0·14	0·09	—	0·19	0·65	0·23
6.9.1926.....	—	2·5	3·0	—	2·0	0·30	—	0·10	0·09	—	0·42	0·91	0·19
17.9.1926.....	—	3·7	1·7	—	0·9	0·23	—	0·26	0·10	—	0·18	0·77	0·36
28.9.1926.....	0·2	1·6	0·8	—	0·6	0·19	0·09	0·10	0·02	—	0·10	0·50	0·21
11.10.1926.....	0·2	2·2	2·2	—	0·9	0·16	0·08	0·11	0·10	—	0·14	0·59	0·29
28.10.1926.....	0·7	4·0	2·0	—	1·2	0·08	0·16	0·12	0·14	—	0·17	0·67	0·42
8.11.1926.....	2·5	7·1	2·1	—	2·1	0·11	0·63	0·36	0·07	—	0·23	1·40	1·06
18.11.1926.....	1·0	2·0	0·3	—	1·5	0·10	0·26	0·10	0·01	—	0·15	0·62	0·37
17.1.1927.....	2·2	1·3	0·2	—	0·9	0·08	0·33	0·05	0·02	—	0·07	0·55	0·40
14.2.1927.....	5·2	3·0	2·7	1·8	1·6	0·11	0·78	0·12	0·32	0·38	0·21	1·92	1·59
28.2.1927.....	3·1	1·0	2·3	1·6	1·0	0·07	0·43	0·06	0·16	0·34	0·08	1·14	0·99
14.3.1927.....	3·2	1·9	2·0	1·0	0·8	0·14	0·38	0·08	0·14	0·17	0·10	1·01	0·77
16.4.1927.....	2·0	2·1	3·3	1·0	0·7	0·15	0·34	0·15	0·10	0·15	0·11	1·00	0·74
25.4.1927.....	1·9	3·8	3·3	0·7	0·9	0·14	0·25	0·38	0·13	0·08	0·12	1·10	0·84
16.5.1927.....	—	4·8	2·0	0·1	0·8	0·21	—	0·44	0·14	—	0·18	0·97	0·58
6.6.1927.....	—	4·4	2·1	—	1·8	0·25	—	0·53	0·15	—	0·36	1·29	0·68
28.6.1927.....	—	5·3	2·6	—	1·3	0·15	—	0·53	0·13	—	0·17	0·98	0·66
18.7.1927.....	—	2·1	1·2	—	0·8	0·21	—	0·13	0·03	—	0·12	0·49	0·16
8.8.1927.....	—	3·4	1·4	—	0·8	0·19	—	0·20	0·10	—	0·11	0·60	0·30
24.8.1927.....	—	3·2	2·2	—	1·6	0·25	—	0·19	0·11	—	0·37	0·92	0·30
14.9.1927.....	—	2·3	1·0	—	0·7	0·22	—	0·18	0·05	—	0·13	0·58	0·23
29.9.1927.....	0·2	1·5	1·6	—	0·7	0·20	0·10	0·07	0·07	—	0·11	0·55	0·24

TABLE 26.—*Harpechloa capensis*. Ermelo, B camp.

Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root = 1.					Amount of P_2O_5 in the Different Organs.						Total P_2O_5.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
12.10.1926.....	1·7	3·1	0·1	—	4·0	0·13	0·24	0·06	—	—	0·28	0·71	0·30
25.10.1926.....	1·7	4·5	1·3	—	3·3	0·06	0·30	0·09	0·11	—	0·23	0·79	0·50
8.11.1926.....	2·2	4·2	0·7	—	1·8	0·09	0·44	0·13	0·02	—	0·20	0·88	0·59
29.12.1926.....	3·1	2·5	1·0	0·7	3·6	0·05	0·47	0·08	0·09	0·19	0·18	1·06	0·83
17.1.1927.....	2·9	3·1	1·0	0·9	2·5	0·03	0·41	0·12	0·07	0·22	0·15	1·00	0·82
31.1.1927.....	3·6	3·9	1·8	0·9	2·3	0·05	0·47	0·16	0·13	0·13	0·16	1·10	0·89
14.2.1927.....	2·9	2·5	1·5	0·5	2·0	0·05	0·61	0·13	0·03	0·07	0·14	1·03	0·84
28.2.1927.....	2·3	2·1	1·4	0·5	1·4	0·08	0·32	0·08	0·13	0·05	0·14	0·80	0·58
14.3.1927.....	3·0	3·4	1·0	0·5	2·8	0·05	0·33	0·07	0·02	0·15	0·14	0·76	0·57
6.4.1927.....	2·6	5·6	1·8	0·3	3·1	0·06	0·42	0·17	0·07	0·05	0·19	0·96	0·71
25.4.1927.....	0·7	2·5	1·0	0·2	3·2	0·07	0·07	0·07	0·02	—	0·19	0·42	0·16
16.5.1927.....	1·7	6·3	1·0	—	3·1	0·12	0·15	0·19	0·02	—	0·25	0·73	0·36
6.6.1927.....	0·1	3·4	1·5	0·1	2·0	0·16	0·02	0·14	0·04	—	0·18	0·54	0·20
28.6.1927.....	0·2	5·5	1·5	—	2·0	0·09	0·03	0·22	0·03	—	0·22	0·59	0·28
18.7.1927.....	—	6·3	1·1	—	3·0	0·09	—	0·25	0·03	—	0·33	0·70	0·28
8.8.1927.....	0·1	3·7	0·9	—	2·0	0·11	0·02	0·15	0·02	—	0·22	0·52	0·19
24.8.1927.....	0·1	6·5	0·9	—	2·3	0·12	0·03	0·26	0·02	—	0·35	0·88	0·41
14.9.1927.....	0·2	5·3	1·1	—	2·4	0·09	0·05	0·16	0·02	—	0·19	0·51	0·23
29.9.9127.....	0·1	1·3	0·6	—	1·8	0·12	0·04	0·04	—	—	0·20	0·40	0·08

TABLE 27.—*Hyparrhenia Buchanani*. Ermelo, O camp.
Absolute amount of phosphorus, in grams phosphoric oxide, in an ideal plant corresponding to 100 gm. roots.

Date.	Relation of the Different Organs. Root=1.					Amount of P_2O_5 in the Different Organs.						Total P_2O_5.	
	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	Roots.	Green Leaves.	Brown Leaves.	Haulms.	Spikes.	Root-neck.	In the Whole Plant.	In the Aerial Parts.
23.8.1926	0·15	3·5	6·5	—	2·0	0·13	0·06	0·32	0·39	—	0·24	1·14	0·77
3.9.1926	0·1	3·2	4·8	—	2·9	0·14	0·02	0·26	0·29	—	0·35	1·06	0·57
16.9.1926	0·4	5·7	4·9	—	3·1	0·13	0·18	0·46	0·20	—	0·46	1·43	0·84
27.9.1926	0·2	3·8	5·1	—	2·6	0·12	0·10	0·34	0·31	—	0·31	1·18	0·75
13.10.1926	0·6	5·0	5·0	—	3·7	0·09	0·17	0·30	0·20	—	0·30	1·06	0·67
25.10.1926	1·7	5·7	3·6	0·5	3·4	0·09	0·37	0·40	0·11	0·03	0·34	1·34	0·91
10.11.1926	1·6	6·2	2·9	0·15	2·1	0·12	0·40	0·37	0·12	0·01	0·27	1·29	0·90
23.11.1926	1·0	3·8	3·1	—	2·8	0·10	0·25	0·27	0·12	—	0·36	1·10	0·64
13.12.1926	2·1	4·9	4·5	—	2·3	0·15	0·92	0·54	0·36	—	0·37	2·34	1·82
4.1.1927	1·6	2·9	2·5	—	3·1	0·09	0·30	0·25	0·10	—	0·31	1·05	0·65
18.1.1927	2·8	2·5	1·5	—	1·8	0·10	0·50	0·18	0·06	—	0·22	1·06	0·74
1.2.1927	3·7	3·4	2·5	0·1	2·3	0·07	0·52	0·14	0·11	—	0·18	1·02	0·77
18.2.1927	4·9	2·4	3·0	0·3	2·0	0·09	1·03	0·19	0·18	0·08	0·22	1·79	1·48
1.3.1927	5·2	3·7	3·9	0·2	2·4	0·07	0·88	0·30	0·20	0·05	0·36	1·86	1·43
15.3.1927	2·8	2·4	3·0	0·3	2·2	0·04	0·98	0·22	0·19	0·11	0·33	1·88	1·51
7.4.1927	2·1	2·2	2·7	0·5	1·3	0·11	0·61	0·20	0·29	0·18	0·17	1·55	1·27
26.4.1927	2·6	2·8	5·5	0·4	2·5	0·13	0·70	0·22	0·17	0·10	0·35	1·67	1·19
17.5.1927	0·4	3·9	4·0	—	1·7	0·14	0·12	0·24	0·23	—	0·27	1·00	0·59
7.6.1927	0·5	2·4	4·4	—	2·0	0·14	0·17	0·24	0·43	—	0·37	1·30	0·84
28.6.1927	0·5	2·6	3·3	—	2·9	0·14	0·17	0·29	0·35	—	0·46	1·41	0·81
19.7.1927	0·4	3·0	2·7	—	2·7	0·13	0·14	0·36	0·25	—	0·59	1·47	0·75
9.8.1927	0·6	6·2	6·8	—	2·4	0·17	0·22	0·62	0·48	—	0·53	2·02	1·32
26.8.1927	1·1	7·8	9·8	—	3·7	0·16	0·56	0·94	1·37	—	0·81	3·84	1·87
15.9.1927	0·9	7·1	8·8	—	3·2	0·13	0·44	0·64	0·44	—	0·45	2·10	1·52
30.9.1927	1·4	4·3	3·6	—	2·1	0·16	0·84	0·56	0·43	—	0·53	2·52	1·83

TABLE 28.—*Themeda triandra*. Green and brown leaves. Phosphorus compounds, B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Substance.					
479	15.11.1926	Green, fresh folded	0·285	0·121	0·046	0·106	0·004	0·018
786	19.1.1927	Green	0·196	0·104	0·034	0·078	0·003	0·015
1034	21.2.1927	Green	0·195	0·055	0·056	0·050	0·003	0·010
1095	15.3.1927	Green	0·187	0·095	—	0·010	0·004	0·011
1211	22.4.1927	Green	0·185	0·120	0·033	0·077	0·005	0·004
1212	22.4.1927	Brown	0·077	0·023	0·009	0·013	0·003	0·005
1367	18.5.1927	Green	0·184	0·056	0·010	0·009	0·005	0·015
1368	18.5.1927	Brown	0·055	0·043	0·009	0·002	—	0·003
1446	16.6.1927	Green	0·184	0·120	—	0·029	—	0·010
1447	16.6.1927	Brown	0·057	0·035	0·013	0·012	0·002	0·003
1514	18.7.1927	Brown	0·054	0·044	0·012	0·000	0·001	0·003
1630	15.8.1927	Brown	0·061	0·057	0·009	0·000	0·002	0·005
1828	18.10.1927	Brown	0·059	0·019	0·010	0·000	0·002	0·006
1827	18.10.1927	Green	0·330	0·200	0·087	0·027	0·004	0·012
1854	15.11.1927	Green	0·238	0·146	0·045	0·009	0·008	0·005
1855	15.11.1927	Brown	0·052	0·011	0·003	0·008	0·002	0·004
1872	16.12.1927	Green	0·316	0·109	0·054	Trace.	0·004	0·005
1873	16.12.1927	Brown	0·068	0·019	0·016	0·000	0·002	0·004
1890	16.1.1928	Green	0·233	0·120	0·073	0·010	0·006	0·006
1891	16.1.1928	Brown	0·068	0·016	0·006	0·000	0·002	0·005
1942	16.2.1928	Green	0·240	0·093	0·040	0·000	0·004	0·009
1943	16.2.1928	Brown	0·061	0·016	0·016	0·000	0·002	0·005
1998	16.3.1928	Green leaves	0·265	0·191	0·125	0·000	0·008	0·006
1999	16.3.1928	Brown	0·092	0·050	0·028	0·000	0·004	0·006

TABLE 29.—*Themeda triandra*. Haulms and spikes. Phosphorus compounds. B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
859	2.2.1927	Young haulms	0·189	0·119	0·031	0·040	0·002	0·007
1035	21.2.1927	Old haulms	0·052	0·022	0·008	0·008	0·001	0·004
1210	22.4.1927	Old haulms and spikes	0·060	0·038	0·038	0·003	—	0·004
1369	18.5.1927	Old haulms and spikes	0·046	0·025	0·023	0·013	—	0·006
1448	16.6.1927	Old haulms	0·038	0·050	0·012	0·014	—	0·004
1569	18.7.1927	Haulms and empty spikes	0·037	0·026	0·008	0·000	—	0·003
1633	15.8.1927	Old haulms	0·053	0·034	0·015	0·009	—	0·004
1829	18.10.1927	Young haulms and spikes	0·272	0·081	—	0·000	—	0·018
1830	18.10.1927	Old haulms	0·024	0·015	—	0·000	—	0·004
1853	15.11.1927	New spikes	0·397	0·214	0·068	0·051	0·004	0·007
1856	15.11.1927	New haulms	0·123	0·084	0·028	0·016	0·003	0·008
1871	16.12.1927	Ears	0·296	0·135	0·045	0·025	—	0·008
1874	16.12.1927	New haulms	0·156	0·079	0·049	0·018	0·003	0·007
1889	16.1.1928	New spikes	0·188	0·068	—	0·013	—	0·007
1892	16.1.1928	New haulms	0·080	0·027	0·011	0·000	Spoilt.	0·006
1941	16.2.1928	Spikes	0·193	0·068	Not enough Material.	0·000	Not enough Material.	0·004
1944	16.2.1928	Haulms	0·065	0·036	0·025	0·025	0·000	0·004
2000	16.3.1928	Haulms	0·067	0·060	0·018	0·000	0·002	0·003

TABLE 30.—*Themeda triandra*. Roots and rootneck. Phosphorus compounds, B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
1036	21.2.1927	Rootneck	0·105	0·033	0·013	0·007	0·003	0·010
1037	21.2.1927	Roots	0·120	0·094	0·037	0·008	0·007	0·015
1213	22.4.1927	Rootneck	0·156	0·100	0·035	0·011	0·003	0·005
1214	22.4.1927	Roots	0·203	0·105	0·038	0·013	0·004	0·005
1370	18.5.1927	Rootneck	0·196	0·095	0·033	0·005	0·004	0·011
1371	18.5.1927	Roots	0·384	0·121	0·078	0·009	0·004	0·005
1449	16.6.1927	Rootneck	0·167	0·084	0·051	0·000	0·004	0·008
1450	16.6.1927	Roots	0·224	0·104	0·032	0·000	0·003	0·009
1517	18.7.1927	Roots	0·158	0·085	0·042	0·008	0·002	0·005
1631	15.8.1927	Roots	0·211	0·141	0·050	0·009	0·009	0·010
1632	15.8.1927	Rootneck	0·189	0·110	0·052	0·000	0·009	0·005
1831	18.10.1927	Rootneck	0·123	0·057	0·046	0·000	0·003	0·006
1832	18.10.1927	Roots	0·171	0·080	0·024	0·000	0·002	0·004
1857	15.11.1927	Rootneck	0·114	0·060	0·040	0·000	0·000	0·007
1858	15.11.1927	Roots	0·096	0·067	0·031	0·000	0·003	0·004
1875	16.12.1927	Rootneck	0·123	0·056	0·027	0·013	0·002	0·008
1876	16.12.1927	Roots	0·113	0·052	0·023	0·000	0·002	0·005
1893	16.1.1928	Rootneck	0·099	0·042	0·030	0·000	0·003	0·005
1894	16.1.1928	Roots	0·071	0·045	0·019	0·000	0·002	0·007
1946	16.2.1928	Roots	0·123	0·062	0·044	0·000	0·001	0·005
1945	16.2.1928	Rootneck	0·115	0·048	0·027	0·000	0·001	0·004
2002	16.3.1928	Roots	0·187	0·131	0·115	0·000	0·005	0·005
2001	16.3.1928	Rootneck	0·164	0·104	0·058	0·000	0·006	0·003

TABLE 31.—*Digitaria tricholaenoides*. Leaves. Phosphorus compounds, X camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
1695	1.9.1927	Brown leaves *	0·091	0·053	0·036	0·013	0·003	0·006
1757	22.9.1927	Green leaves	0·524	0·414	0·156	0·040	0·024	0·039
1833	24.10.1927	Green leaves	0·360	0·172	0·071	0·055	0·008	0·017
1834	24.10.1927	Brown leaves	0·100	0·029	0·011	0·007	—	0·004
1860	21.11.1927	Green leaves	0·304	0·170	0·063	0·057	0·006	0·014
1878	20.12.1927	Green leaves	0·245	0·137	0·080	0·115	0·008	0·007
1895	23.1.1928	Green leaves	0·236	0·154	0·050	0·090	0·006	0·005
1947	20.2.1928	Green leaves	0·212	0·113	0·050	0·035	0·006	0·006
1948	20.2.1928	Brown leaves	0·109	0·026	0·006	0·008	0·002	0·006
2003	20.3.1928	Green leaves	0·214	0·139	0·096	0·019	0·011	0·010
2004	20.3.1928	Old leaves	0·090	0·046	0·029	0·000	0·005	0·006
2020	20.4.1928	Green leaves	0·258	0·213	0·186	0·018	0·014	0·006
2021	20.4.1928	Old leaves	0·118	0·075	0·068	0·000	0·005	0·005
2043	21.5.1928	Green leaves†	0·204	0·151	0·097	‡	0·017	0·003
2044	21.5.1928	Old leaves	0·083	0·053	0·030	0·007	0·004	0·005
2047	20.6.1928	Old leaves	0·082	0·051	0·018	0·000	0·005	0·005
2057	20.7.1928	Old leaves	0·082	0·057	0·031	0·007	0·004	0·006

* Camp burned the same day.
† Green leaves scarce.
‡ Too little for determination.

TABLE 32.—*Digitaria tricholaenoides*. Root and Rootneck. Phosphorus compounds. X camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
1697	1.9.1927	Rootneck	0·143	0·081	0·027	0·000	0·006	0·005
1698	1.9.1927	Roots	0·164	0·092	0·040	0·005	0·003	0·009
1758	22.9.1927	Rootneck	0·137	0·069	0·030	0·000	0·002	0·009
1759	22.9.1927	Roots	0·141	0·079	0·025	0·000	0·004	0·008
1836	24.10.1927	Roots	0·134	0·063	0·051	0·000	—	0·007
1835	24.10.1927	Rootneck	0·143	0·045	0·026	0·000	0·002	0·012
1862	21.11.1927	Rootneck	0·111	0·050	0·023	0·008	0·002	0·005
1863	21.11.1927	Roots	0·100	0·050	0·024	0·000	0·002	0·005
1880	20.12.1927	Rootneck	0·123	0·056	0·056	0·000	0·001	0·006
1881	20.12.1927	Roots	0·110	0·039	0·023	0·000	0·001	0·006
1897	23.1.1928	Rootneck	0·120	0·046	0·017	0·026	0·001	0·009
1898	23.1.1928	Roots	0·100	0·047	0·032	Trace.	0·001	0·004
1950	20.2.1928	Roots	0·087	0·045	0·031	0·005	0·002	0·005
1949	20.2.1928	Rootneck	0·113	0·050	0·023	0·000	0·002	0·007
2006	20.3.1928	Roots	0·120	0·079	0·059	0·000	0·004	0·005
2005	20.3.1928	Rootneck	0·128	0·098	0·068	0·000	0·004	0·002
2023	20·4·1928	Roots	0·206	0·159	0·129	0·000	0·007	0·005
2022	20.4.1928	Rootneck	0·162	0·135	0·117	0·000	0·005	0·002
2046	21·5·1928	Roots	0·208	0·136	0·118	0·009	0·010	0·005
2045	21.5.1928	Rootneck	0·168	0·126	0·118	0·000	0·008	0·002
2049	20.6.1928	Roots	0·186	0·137	0·128	0·000	0·011	0·005
2048	20.6.1928	Rootneck	0·147	0·115	0·048	0·007	0·007	0·002
2059	20.7.1928	Roots	0·200	0·142	0·083	0·006	0·009	0·005
2058	20.7.1928	Rootneck	0·148	0·130	0·110	0·007	0·006	0·002

TABLE 33.—*Digitaria tricholaenoides*. Spikes and haulms. Phosphorus compounds. X camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
1859	21.11.1927	Spikes with seeds	0·456	0·334	0·254	0·057	0·013	0·019
1861	21.11.1927	Haulms	0·272	0·183	0·081	0·030	0·008	0·009
1877	20.12.1927	Spikes	0·220	—	—	—	—	—
1879	20.12.1927	Haulms	0·204	0·183	0·081	0·030	0·008	0·009
1887	6.1.1928	Green haulms	0·145	0·090	0·014	0·007	0·005	0·006
1888	6.1.1928	Spikes, seeds fallen	0·145	0·089	—	0·011	—	0·007

TABLE 34.—*Monocymbium cerisaeforme*. Green and brown leaves. Phosphorus compounds, B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
625	23.12.1926	Green leaves	0·235	0·061	0·004	0·070	Trace.	0·021
860	2.2.1927	Green	0·159	0·072	0·008	0·060	0·002	0·013
1128	30.3.1927	Green	0·133	0·046	0·011	0·040	0·003	0·012
1298	29.4.1927	Green	0·136	0·072	0·024	0·081	0·001	0·004
1299	29.4.1927	Old	0·061	0·023	0·003	0·015	0·001	0·005
1373	31.5.1927	Brown	0·071	0·037	0·018	0·027	0·001	0·005
1510	8.7.1927	Brown	0·067	0·030	0·018	0·024	0·002	0·005
1572	1.8.1927	Green	0·240	0·137	—	—	—	0·017
1572A	1.8.1927	Brown	0·045	0·021	0·005	0·011	0·002	0·005
1686	30.8.1927	Brown	0·057	0·020	0·008	0·000	0·002	0·007
1685	30.8.1927	Green	None.	—	—	—	—	—
1821	4.10.1927	Very small, green	0·528	0·250	0·078	0·033	0·016	0·036
1822	4.10.1927	Brown	0·056	0·020	0·006	0·008	0·002	0·006
1844	4.11.1927	Green	0·387	0·240	0·120	0·068	0·013	0·027
1845	4.11.1927	Brown	0·048	0·018	0·008	0·000	0·001	0·006
1864	30.11.1927	Green	0·260	0·192	0·137	0·040	0·011	0·015
1865	30.11.1927	Brown	0·061	0·022	0·002	0·010	0·002	0·006
1882	27.12.1927	Green	0·194	0·148	0·101	0·032	0·005	0·012
1883	27.12.1927	Brown	0·070	0·020	0·020	0·024	0·002	0·006
1916	31.1.1928	Green	0·197	0·161	0·061	0·014	0·001	0·012
1917	31.1.1928	Brown	0·065	0·014	0·002	0·005	0·001	0·001
1974	29.2.1928	Green	0·170	0·075	0·026	0·000	0·004	0·012
1975	29.2.1928	Brown	0·060	0·015	0·013	0·000	0·002	0·007

TABLE 35.—*Monocymbium cerisaeforme*. Haulms and spikes. Phosphorus compounds, B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
1127	30.3.1927	Spikes and haulms, no seeds	0·090	0·058	0·006	0·076	0·002	0·008
1300	29.4.1927	Haulms	0·154	0·036	0·019	0·004	0·001	0·001
1297	29.4.1927	Spikes	0·101	0·073	—	0·027	—	0·006
1374	31·5·1927	Old haulms and spikes	0·030	0·035	0·018	0·027	0·001	0·004
1511	8.7.1927	Old haulms	0·040	0·024	0·024	0·020	0·001	0·003
1574	1.8.1927	Old haulms	0·034	0·022	0·018	0·004	0·002	0·002
1689	30.8.1927	Old haulms	0·044	0·019	0·012	0·000	0·001	0·002
1823	4.10.1927	Old haulms	0·033	0·027	0·009	0·000	0·001	0·006
1850	4.11.1927	Old haulms	0·037	0·024	0·008	0·000	—	0·004
1870	30.11.1927	Old haulms	0·026	*	*	*	*	*
1886	27.12.1927	New haulms	0·192	*	*	*	*	*
1915	31.1.1928	Spikes	0·252	0·164	0·057	0·000	0·002	0·005
1918	31.1.1928	Haulms	0·105	0·066	0·009	0·000	0·002	0·006
1973	29.2.1928	Spikes	0·214	0·099	0·029	0·000	0·004	0·008
1976	29.2.1928	Haulms	0·076	0·047	0·023	0·000	0·002	0·005

* Too little material.

TABLE 36.—*Monocymbium cerisaeforme*. Roots and rootneck. Phosphorus compounds, B camp.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
624	13.12.1926	Roots	0·097	0·057	0·018	0·008	—	0·009
1129	30.3.1927	Rootneck	0·096	0·026	0·006	0·005	0·003	0·025
1130	30.3.1927	Roots	0·095	0·039	0·009	0·003	0·001	0·007
1302	29.4.1927	Roots	0·189	0·099	0·036	0·008	0·003	0·008
1301	29.4.1927	Rootneck	0·154	0·086	0·045	Trace.	0·002	0·006
1375	31.5.1927	Rootneck	0·172	0·098	0·038	0·000	0·002	0·004
1376	31.5.1927	Roots	0·205	0·115	0·066	0·007	0·004	0·007
1512	18.6.1927	Rootneck	0·123	0·053	0·045	0·006	0·002	0·006
1513	18.6.1927	Roots	0·156	0·120	0·030	0·004	0·003	0·008
1573	1.7.1927	Roots	0·200	0·126	0·026	0·000	0·003	0·008
1575	1.7.1927	Rootneck	0·167	0·125	0·084	0·013	0·002	0·004
1688	30.7.1927	Rootneck	0·151	0·085	0·085	0·000	0·002	0·009
1687	30.7.1827	Roots	0·200	0·131	0·065	0·000	0·004	0·010
1824	4.10.1927	Rootneck	0·130	0·091	0·030	0·000	0·002	0·009
1825	4.10.1927	Roots	0·168	0·111	0·039	0·000	0·003	0·008
1851	4.11.1927	Rootneck	0·141	0·058	0·048	0·000	0·003	0·008
1852	4.11.1927	Roots	0·144	0·096	0·081	0·000	0·003	0·006
1866	30.11.1927	Rootneck	0·098	0·053	0·032	0·000	0·002	0·004
1867	30.11.1927	Roots	0·097	0·067	0·034	0·007	0·001	0·004
1883	27.12.1927	Rootneck	0·086	0·064	0·043	0·000	0·003	0·005
1884	27.12.1927	Roots	0·070	0·026	0·022	0·007	0·002	0·004
1920	31.1.1928	Roots	0·068	0·028	0·020	0·000	0·001	0·003
1919	31.1.1928	Rootneck	0·106	0·033	0·007	0·000	0·001	0·005
1978	29.2.1928	Roots	0·079	0·050	0·018	0·000	0·001	0·006
1977	29.2.1928	Rootneck	0·128	0·069	0·050	0·000	0·001	0·007

TABLE 37.—*Zea Mais*. Leaves. Phosphorus compounds.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia. Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
958	1.2.1927	Green leaves	0·290	0·118	0·109	0·164	0·004	0·029
966	28.2.1927	Green leaves	0·294	0·161	0·090	0·230	0·002	0·015
1118	28.3.1927	Green leaves	0·246	0·246	0·064	0·068	0·004	0·024
1123	30.3.1927	Green leaves fertilized	0·202	0·161	0·065	0·149	0·004	0·024
1292	29.4.1927	Green leaves	0·210	0·127	0·028	0·043	0·010	0·019

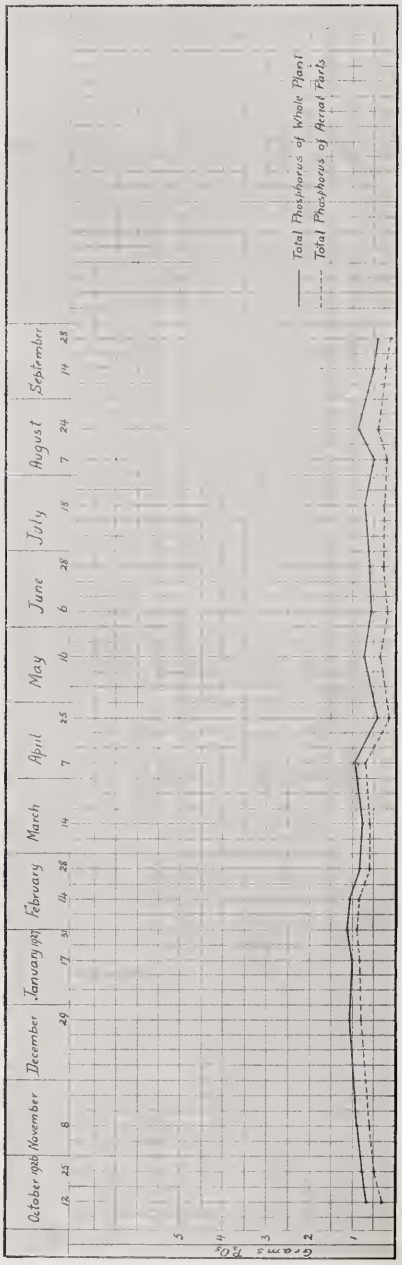
TABLE 38.—*Zea Mais.* Stalks, covering sheets. Middle of cobs. Roots and rootneck. Phosphorus compounds.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia. Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Material.					
959	1.2.1927	Stalks	0·220	0·109	0·080	0·040	0·003	0·018
960A	1.2.1927	Leaves round cobs	0·273	0·182	0·046	0·054	0·004	0·006
964	28.2.1927	Sheets and centres	0·263	0·263	0·070	0·024	0·004	0·012
965	28.2.1927	Stalks	0·135	0·092	0·031	0·041	0·002	0·004
1117	28.3.1927	Cobs	0·167	0·086	0·040	0·013	0·004	0·007
1119	28.3.1927	Roots	0·140	0·078	0·032	0·010	0·002	0·006
1122	30.3.1927	Cobs, centres	0·106	0·081	0·030	0·054	0·003	0·019
1124	30.3.1927	Stalks of fertilized mealies	0·236	0·057	0·022	0·027	0·002	0·007
1125	30.3.1927	Rootneck, fertilized mais	0·100	0·058	0·020	0·009	0·002	0·005
1126	30.3.1927	Roots, fertilized mais	0·103	0·043	0·020	0·013	0·001	0·005
1291	29.4.1927	Coverings, centres of cobs	0·093	0·035	0·023	0·000	0·002	0·005
1293	29.4.1927	Stalks	0·119	0·060	0·034	0·011	0·002	0·012
1294	29.4.1927	Rootneck	0·094	0·033	0·019	0·022	0·002	0·002
1295	29.4.1927	Roots	0·105	0·029	0·023	0·020	0·001	0·010

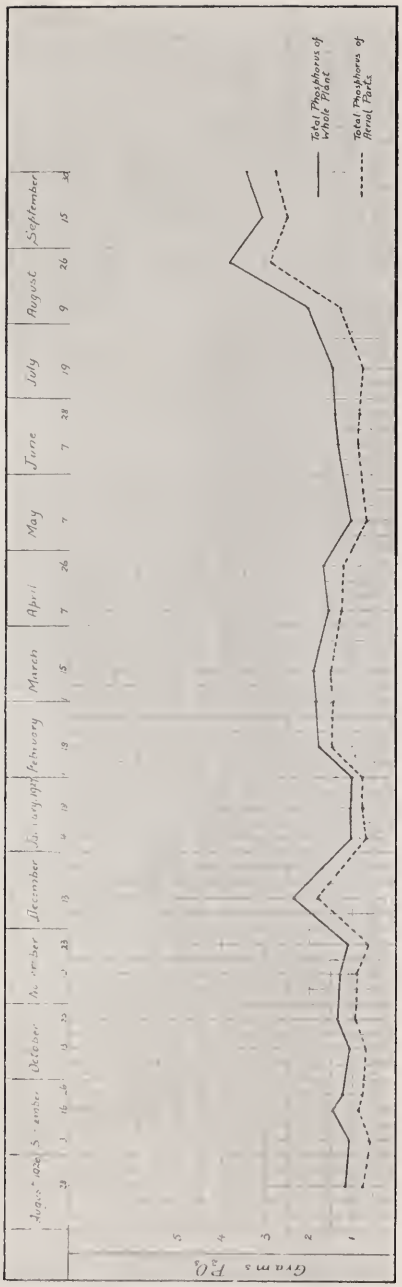
TABLE 39.—*Zea Mais Ears.* Fertilized and unfertilized. Phosphorus content.

No.	Date.	Remarks.	Total P_2O_5.	Water Soluble P_2O_5.	P_2O_5 Precipitated with Magnesia. Mixture.	P_2O_5 of Phytin.	P_2O_5 of Lecithin.	P_2O_5 of Nucleo-proteids.
			In % of Air Dry Substance.					
960	1.2.1927	Very young cobs	0·423	0·273	0·046	0·101	Trace.	0·060
962	28.2.1927	♂spikes flowering	0·212	0·144	0·044	0·071	0·004	0·030
1116	28.3.1927	Seeds soft	0·463	0·250	0·118	0·251	0·003	0·021
1127	30.3.1927	Male spikes fertilized	0·211	0·092	0·040	0·098	0·004	0·024
1121	30.3.1927	Cobs and grains fertilized	0·504	0·347	0·282	0·360	Trace.	0·028
1290	29.4.1927	Ripe grains	0·524	0·312	0·228	0·395	Trace.	0·034
1296	29.4.1927	Male spikes old and decaying	0·183	0·090	—	0·004	—	0·010

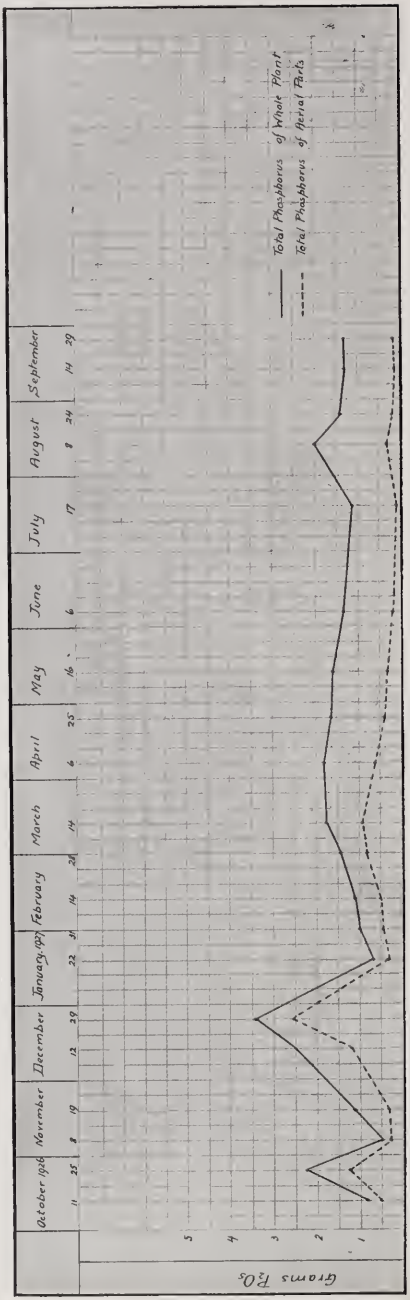
GRAPH 1.—*Harpechloa capensis*: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots. (In gr. P_2O_5.)



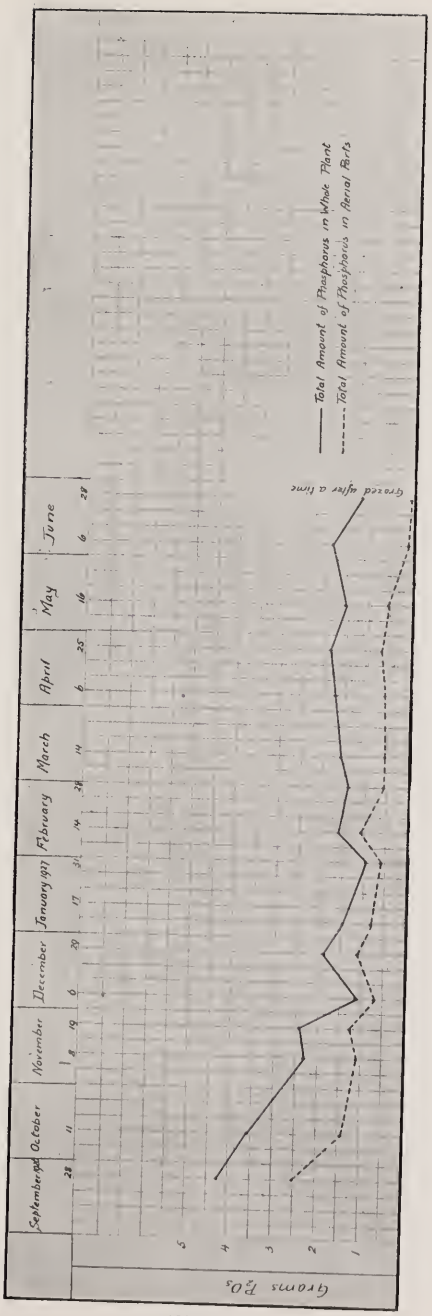
GRAPH 2.—*Hyparrhenia Buchanani*: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots.
(In gr. P_2O_5.)



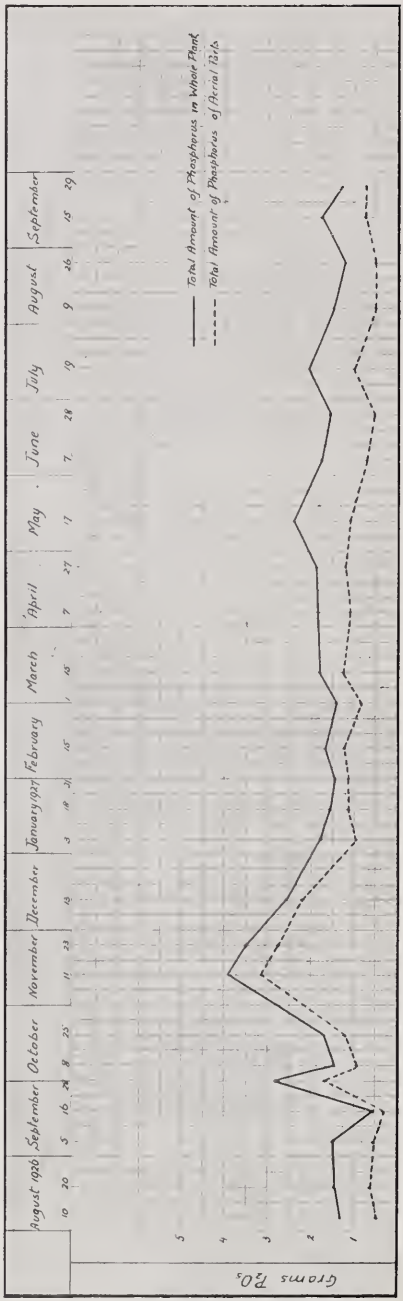
GRAPH 3.—*Digitaria tricholaenoides*: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots.
(In gr. P_2O_5.)



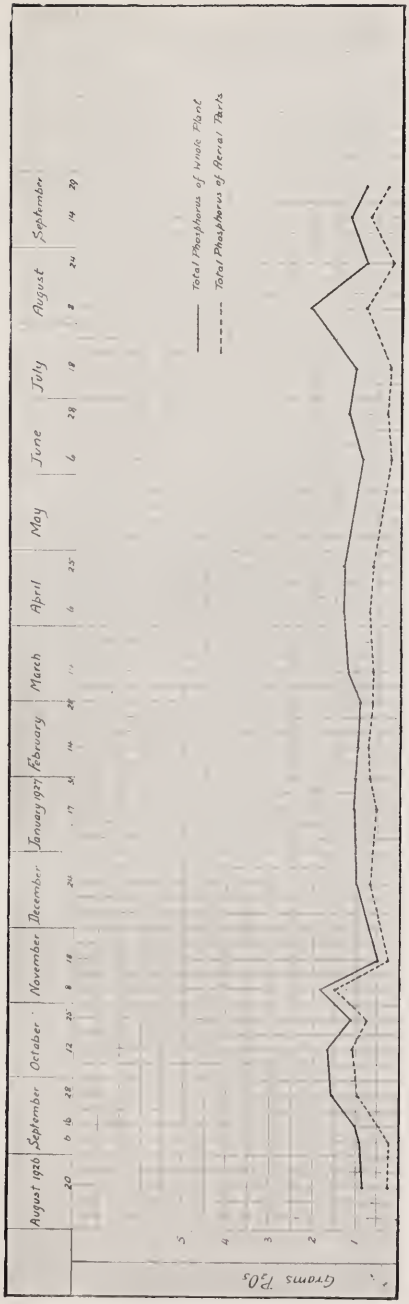
GRAPH 4.—*Pennisetum Thunbergii*: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots. (In gr. P_2O_5.)



GRAPH 5.—*Themeda triandra*, O. Camp: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots.
(In gr. P_2O_5.)



GRAPH 6.—*Thevneda triandra*, B. Camp: Total Amount of Phosphorus of a Plant corresponding to 100 gr. Roots.
(In gr. P_2O_5.)



Section IX.

Animal Industry.

A. E. Romyn, F. N. Bonsma, C. A. Murray, and G. N. Murray — Investigations into the Production of Bacon.

Investigations into the Production of Bacon.

By A. E. ROMYN, M.Sc., Ph.D.; F. N. BONSMA, B.Sc.;* C. A. MURRAY, B.Sc.; G. N. MURRAY, B.Sc.; Division of Veterinary Services and Animal Industry.

INTRODUCTION.

Conditions in the Union of South Africa are on the whole well adapted to the rearing and fattening of pigs. The local demand for bacon, however, is relatively small and it has been realised from the outset that, unless a satisfactory market for South African products can be found overseas, little will be gained from an expansion of the local industry beyond its present moderate dimensions (Romyn, 1929). The sporadic attempts at export made so far have not been encouraging. There is evidence, however, to show that there are possibilities of a profitable market overseas if the proper methods of production are followed in the Union.

In 1926 a series of trial exports from representative bacon factories in the Union were made to Great Britain. In these consignments each factory sent forward what were considered typical samples of the bulk of the supplies and no attempt was made to send specially selected sides. The shipments suffered somewhat in transit owing to the impracticability of obtaining proper refrigeration facilities for the small quantities exported. The reports received on the bacon indicated that the Union can produce bacon comparable to good Danish but that the sides exported in general lacked—

(*a*) length and uniformity;

(*b*) firmness.

These undesirable characteristics were ascribed to—

(*a*) excessive maize feeding;

(*b*) an unsuitable type of pig. (Dimond, 1926.)

The chief grain grown in the Union is maize, and excessive maize feeding, as understood by the English bacon curer, is more or less unavoidable in most sections of the country. By careful breeding methods it should, however, be possible to meet the demands of the overseas market in regard to the type of pig.

It has been indicated in a recent report of the Empire Marketing Board (Imperial Economic Committee, 1929) that South African producers would be well advised to give up their attempts to produce a Wiltshire type of bacon and to turn their attention rather to the

* Since resigned from the Department of Agriculture.

production of a fatter type of bacon, which the Board considers the more logical product of a maize ration. At present there is a satisfactory market for this type of bacon in the North of England. The change to a fatter type pig entails a reversal of the present breeding policy and is viewed with misgiving in some quarters as likely to bring the South African producer into direct competition with the maize producing areas of North and South America.

As far as South Africa is concerned reliable data bearing on the problem are scanty. The investigations summarised in this report were undertaken with the object of giving this Department some definite information on the question. It was hoped that the results of this work will help to show the best line of action to be followed by pig producers in the Union.

The experiments described were carried out at the Cedara and Potchefstroom Schools of Agriculture. The purpose of the investigation has been set out as being:—

> "To determine whether it is possible by careful selection to produce a satisfactory Wiltshire side on a ration consisting mostly of maize, or on a maize ration modified only to an extent justified by local economic conditions."

Though valuable work of a similar nature was carried out at the Experiment Station at Elsenburg during the period 1920-24, maize formed only a small portion of the rations used and, unfortunately, the results do not bear directly on the problem under investigation. (Morkel and Johnston, 1926.)

DESCRIPTION OF THE EXPERIMENTS.

The Pigs Used.

The relevant data on 329 pigs have been summarised in this report. The experiments have been divided into two series. Series 1, covers the period from November, 1926, to September, 1928, and series 2, the period from September, 1928, to November, 1929.

The trials were carried out with cross-bred pigs. The sows used were pure-bred Large Blacks of good conformation but showing some variation in type. In successive trials they were bred to Middle White, Tamworth and Large White Boars. In most cases the same sows were used throughout the experiments. At each institution, therefore, the results of one trial are fairly comparable with the next.

Management of the Pigs.

Prior, and subsequent to, farrowing the sows received the same treatment each year. The dry sows were run on any pasture available and were given a small amount of supplementary feed when necessary, generally maize and meat meal. At Cedara the animals were pastured on Kikuyu grass during the summer, and artichokes during the winter. At Potchefstroom the dry sows were run on a mixture of paspalum and veld grass or veld grass alone.

The sows were removed to fresh paddocks or to styes for farrowing. After farrowing the grain ration was increased according to the usual practice. Generally it was modified also by the addition of separated milk and sometimes by wheaten pollard or bran as well.

The young pigs were weaned at from 8-11 weeks of age and divided into comparable lots taking into consideration age, development and breeding. At Cedara the baconers were all run on Kikuyu pasture. The quality of this pasture varied with the season of the year. At Potchefstroom, where good pasture was generally not available, the usual praetice was to feed the baconers in cement styes or dry lots.

The Rations Used.

Series 1.

In the first series of experiments the pigs were divided into three groups:—

Group 1. Received a ration of ground maize 90 parts and meat meal 10 parts by weight.

Group 2. Received a ration of ground maize 90 parts and meat meal 10 parts until the pigs reached a live weight of approximately 130 lb. when the ration was changed to one of ground maize 100 parts, Pollard * 100 parts and ground barley 100 parts by weight.

Group 3. Received a ration of ground maize 100 parts, Pollard 100 parts and ground barley 100 parts by weight throughout.

At Cedara all the baconers had access to similar paddocks of Kikuyu pasture. At Potchefstroom the pigs were kept in styes and the green feed was hand-fed.

The pigs were fed these rations twice daily in such amounts as they would clean up in 15-20 minutes. In some cases small quantities of separated milk (up to ½ gal. per day) were fed when available. At Cedara a mixture of equal parts meatmeal and bloodmeal was used instead of the meatmeal alone. In compiling this report the results on meatmeal, meat and bloodmeal, have not been kept separate. A mineral mixture containing sterilised bonemeal and salt was available to all the lots.

Series 2.

From the experience gained in the first series of trials the rations in the second series were changed to those given below. The reasons for the alterations are outlined in a later section of this report.

Group 1. Pigs fed in dry lot or stye. No separated milk.

Lot 1. Ground maize 90 parts; meatmeal 10 parts by weight.

Lot 2. Ground maize 45 parts; ground barley 45 parts; meatmeal 10 parts by weight.

* Wheat middlings.

Lot 3. Ground maize 45 parts; ground kaffircorn 45 parts; meatmeal 10 parts by weight.

Group 2. Pigs full fed on Kikuyu pasture. Separated milk in varying quantities.

Lot 1. Ground maize 90 parts; meatmeal 10 parts by weight.

Lot 2. Ground maize 45 parts; ground kaffircorn 45 parts; meatmeal 10 parts by weight.

Group 3. Pigs fed two-thirds the grain ration of group 2 on Kikuyu or lucerne pasture. A limited ration of separated milk was fed in the case of the pigs on Kikuyu pasture.

Lot 1. Shelled maize—lucerne pasture.

Lot 2. Ground maize 90 parts; meatmeal 10 parts by weight; Kikuyu pasture.

At Cedara a mixture of equal parts of meatmeal and bloodmeal was used instead of meatmeal alone. Similarly to Series 1, the meatmeal and blood and meatmeal results have not been kept separate. A mineral mixture containing sterilised bonemeal and salt was fed to all lots.

The Records Kept.

At the commencement of each trial the pigs were weighed at intervals of two weeks. When the pigs approached market weights this interval was shortened to weekly weighings. As the pigs reached a desirable state of finish, a state which usually occurred at from 190-210 lb. live weight depending on the cross used, they were railed to the Farmers' Co-operative Bacon Factory, Estcourt, Natal.

At the factory, after the carcasses had been dressed and the customary weights recorded, each side was graded by the manager of the factory, Mr. Welsh, in co-operation with the officer responsible for the particular experiment. The sides were classified into Nos. 1 and 2 " lean sizable," Nos. 1 and 2 " medium, fat and overfat."

The No. 1 " lean sizable " represents the type of side most in demand on the London market. Though well finished this type of carcass has the thinnest layer of backfat of all grades. It should also show good quailty.

A No. 1 " medium " suits the North of England trade but does not command a ready sale on the London market. This type of carcass is thicker in backfat than the No. 1 " lean sizable." In other respects they are similar.

The No. 2 " lean sizable " and No. 2 " medium " sides represent types similar to their respective No. 1's, but lack somewhat in quality.

The No. 1 " fat," or " stout," carcass is between the No. 1 " medium " and " overfat " in thickness of backfat. This type of carcass is becoming more and more undesirable as the demand for lean bacon increases. These carcasses should also show good quality.

The " overfat " is self-explanatory. Bacon is not made from this grade of side.

Towards the end of the first series of trials, a system of carcass measurements was introduced to supplement the system of grading by inspection. " Refractive Index " determinations were also made to determine the firmness of backfat. The method following in making the refractive index determinations was similar to that used by the United States Department of Agriculture in their " soft pork " investigations. [U.S.D.A. Bul. 1407 (1926), 1492 (1928).]

A DISCUSSION OF RESULTS.

Series 1.

(a) The Rate and Economy of Gains.

The daily gain in live weight and the feed consumption per 100 lb. gain in live weight are shown in Table I.

TABLE I.—The Daily Gain in Live Weight and Feed Consumption per 100 lb. Gain in Live Weight—Series 1.

Ration.	Breed of Pig.	No. of Trials.	No. of Pigs.	Average Initial Weight.	Average Final Weight.	Average Daily Gain.	Concentrates Consumed per 100 lb. Increase in Live Weight.	
							Grain Mixture.	Separated Milk.
				lb.	lb.	lb.	lb.	lb.
Maize–Meatmeal.....	Middle White x Large Black.....	3	25	49·9	196·0	1·31	277	318
	Tamworth x Large Black (1).....	2	23	51·1	194·4	1·47	230	218
	Large White x Large Black.....	1	10	54·0	208·1	1·47	320	217
	Average of all trials.....	—	—	51·6	199·5	1·41	306	221
Maize–Meatmeal, finished on Maize–Pollard–Barley	Middle White x Large Black.....	3	25	55·2	197·6	1·43	309	286
	Tamworth x Large Black (1).....	2	23	51·1	196·5	1·45	318	211
	Large White x Large Black.....	1	10	54·0	209·5	1·40	329	131
	Average of all trials.....	—	—	53·4	201·2	1·42	319	209
Maize–Pollard–Barley.	Middle White x Large Black.....	2	18	54·7	196·6	1·46	302	278
	Tamworth x Large Black (1).....	2	23	49·6	197·6	1·46	316	222
	Large White x Large Black.....	1	10	53·9	203·8	1·34	346	135
	Average of all trials.....	—	—	52·7	199·3	1·42	321	212

(1) 12 pigs in each of the Tamworth x Large Black groups did not have access to pasture.

An inspection of Table I will show that the rate, and feed-cost of gain in live weight were remarkably similar on all the rations. The average daily rate of gain of 1.4 lb. is very satisfactory. Though the three rations were apparently equally efficient from the feeding standpoint, barley and pollard are normally higher in price than maize, and the average cost of concentrates per lb. gain in live weight in the three rations at the prices of feeds current during the experiments worked out at 2s. 9d., 3s. 4d. and 3s. 9d. for rations 1, 2 and 3 respectively. If the question of the final product is left out of consideration, ration 1 proves the most satisfactory from the producer's standpoint.

In these trials the breed of pig has apparently made no difference to the rate or economy of gain.

(*b*) *The Relation between the Ration and the Grade of Side produced.*

The relation between the ration and the grade of side produced is shown in Table II.

TABLE II.—The Grading of the Sides produced on the Different Rations—Series 1.

Ration.	Breed of Pig.	No. of Trials.	No. of Pigs.	Grade of Side Produced.					
				No. 1 Lean Sizable.	No. 2 Lean Sizable.	No. 1 Medium.	No. 2 Medium.	Fat.	Over Fat.
Maize–Meatmeal.....	Middle White x Large Black..	3	25	1	2	5	5	8	4
	Tamworth x Large Black.....	2	23	1	—	22	—	—	—
	Large White x Large Black....	1	10	—	—	10	—	—	—
	Total for all trials.....	6	68	2	2	37	5	8	4
Maize–Meatmeal, finished on Maize–Pollard–Barley	Middle White x Large Black...	3	25	2	1	6	2	6	8
	Tamworth x Large Black.....	2	23	8	—	15	—	—	—
	Large White x Large Black....	1	10	2	—	7	1	—	—
	Total for all trials.....	6	58	12	1	28	3	6	8
Maize–Pollard–Barley	Middle White x Large Black...	2	18	2	—	7	2	3	4
	Tamworth x Large Black.....	2	23	7	1	13	2	—	—
	Large White x Large Black....	1	10	5	—	5	—	—	—
	Total for all trials.....	5	51	14	1	25	4	3	4

(1) *Effect of Breed.*

From an inspection of Table II it appears that the Middle White-Large Black Cross has the tendency to produce sides which are definitely fatter than and further removed from the " lean sizable " standard than those produced by either the Tamworth-Large Black or the Large White-Large Black Cross. This tendency is evidenced on all three rations, the only " fat " and " overfat " sides produced being from the Middle White Cross. The results obtained from the Middle White Cross are in keeping with the practical experience of many farmers and are considered sufficiently conclusive to justify the elimination of this cross from the second series of trials.

(2) *Effect of Feed.*

The results summarised in Table II show an increase in the proportion of " lean sizable " sides in the groups receiving ground barley. Excluding the Middle White Cross, the proportion of lean sizable sides increases from 3 per cent. on ration 1 to approximately 30 per cent. on rations 2 and 3. The numbers involved are too small to make these figures significant but, despite the expense of the feed, the results are considered sufficiently promising to justify a further trial with ground barley in the second series of experiments. Pollard was originally included in these experiments on account of its popularity as a pig feed in the wheat growing sections of the country. The supply of this feed is, however, relatively limited. As there is no particular reason to believe that it would have a hardening effect on the carcass, from practical considerations, the feeding of pollard was discontinued in the second series of trials.

Series 2.

(*a*) *Rate and Economy of Gain.*

The rate and economy of gain shown by the various groups are given in Table III.

TABLE III.—The Daily Gain in Live Weight and Feed Consumption per 100 lb. Gain in Live Weight—Series 2.

Ration.	Breed.	Trial Number.	No. of Pigs.	Initial Weight.	Final Weight.	Average Daily Gain.	Concentrates Consumed per 100 lb. Gain in Live Weight.		Separated Milk.
							Grain Mixture.	Separated Milk.	
				lb.	lb.	lb.	lb.	lb.	
Group 1.—*Dry Lot or Stye, no Separated Milk.*									
Maize–Meatmeal.....	Tamworth x Large Black	1	15	44·0	192	1·0	372	0	Nil.
	Tamworth x Large Black	2	12	38·0	193	1·2	368	0	Nil.
	Average two trials....	—	—	41·0	192·5	1·1	370	0	
Maize – Barley–Meatmeal	Tamworth x Large Black	1	12	39·0	191·0	1·2	378	0	Nil.
Maize – Kaffircorn – Meatmeal	Tamworth x Large Black	1	12	38	192	1·0	411	0	

GROUP 2.—*Full Fed on Kikuyu Pasture, with or without Separated Milk.*									
Maize – Meatmeal, Kikuyu Pasture	Large White x Large Black	1	12	54	206	1·32	321	0	Nil.
	Large White x Large Black	2	12	53	212	1·53	254	614	1 gallon per day throughout.
	Large White x Large Black	3	10	54	208	1·47	320	127	Small quantities when available.
	Large White x Large Black	4	20	56	196	1·60	321	25	Small quantities when available.
	Large White x Large Black	5	12	55	198	1·65	269	570	2 gallons per day throughout.
	Average five trials....	—	—	54·4	204	1·51	297	267	
Maize – Kaffircorn – Meatmeal, Kikuyu Pasture	Large White x Large Black	1	20	56	195	1·65	320	25	Small quantities when available.
GROUP 3.—*Two-thirds Ration on Kikuyu or Lucerne Pasture, with or without Separated Milk.*									
Maize and Lucerne Pasture	Tamworth x Large Black	1	15	43	191	1·1	208	—	Nil.
Maize–Meatmeal and Kikuyu Mixture	Large White x Large Black	1	10	54	213	1·24	287	147	Small quantities when available.
	Average of two trials.	—	—	48·5	202	1·17	248	74	

In the trials in series 2 the Large White-Large Black Cross shows up better than the Tamworth-Large Black. The average daily rate of gain was 1.5 lb. for the Large White Cross as compared with 1.1 lb. for the Tamworth Cross. All the Large White crosses, however, were raised at Cedara and the Tamworth crosses at Potchefstroom. The better rate of gain on the part of the Large Whites can be attributed partly to the better development of the weaners at Cedara and partly to the pasture available.

Where conditions were the same as regards pasture or dry lot the rations used appear to be equally efficient. From a practical standpoint the maize-meatmeal ration again proves the most economical.

(*b*) *The Relation between the Ration and the Grade of Side produced.*

The factory grading of the various sides is given in Table IV.

TABLE IV.—The Grading of the Sides produced on the Different Rations—Series 2.

Ration.	Breed.	Trial Number.	Number of Pigs.	Grade of Side Produced.					
				No. 1 Lean Sizable.	No. 2 Lean Sizable.	No. 1 Medium.	No. 2 Medium.	Fat.	Overfat.
Group 1.—*Dry Lot or Stye, no Separated Milk.*									
Maize–Meatmeal.....	Tamworth x Large Black.....	1	15*	5	—	4	—	4	—
	Tamworth x Large Black.....	2	12	5	—	7	—	—	—
Maize–Barley–Meatmeal	Tamworth x Large Black.....	1	12	6	—	6	—	—	—
Maize – Kaffircorn – Meatmeal	Tamworth x Large Black.....	1	12	3	—	6	—	3	—
Group 2.—*Full Fed on Kikuyu Pasture, with or without Separated Milk.*									
Maize–Meatmeal.....	(1) Large White x Large Black.	1	12	1	4	4	3	—	—
	Large White x Large Black.	2	12	5	—	6	—	1	—
	Large White x Large Black.	3	10	2	—	8	—	—	—
Kikuyu Pasture.....	(1) Large White x Large Black.	4	20	4	3	6	1	6	—
	Large White x Large Black.	5	12	3	3	3	1	—	—
Maize – Kaffircorn – Meatmeal, Kikuyu Pasture	Large White x Large Black....	1	20	7	3	3	—	7	—
Group 3.—*Two-thirds Ration on Kikuyu or Lucerne Pasture, with or without Separated Milk.*									
Maize, Lucerne Pasture	Tamworth x Large Black.....	1	15	10	2	3	—	—	—
Maize – Meatmeal, Kikuyu Pasture	Large White x Large Black....	1	10	5	—	5	—	—	—

* Grading not available on two sides.

(1) *Effect of the Breed.*

As just indicated the results from the Tamworth-Large White Cross are not strictly comparable, but it is interesting to note that 39 per cent. of the Tamworth sides were graded No. 1 "lean sizable" as compared with 26 per cent. of the Large White-Large Black Crosses.

In this series all the sides were measured after slaughter. The most important carcass measurements made are given in Table V.

Typical groups of the pigs used in these experiments

Middle White—Large Black Cross.

Tamworth—Large Black Cross.

Large White—Large Black Cross.

TABLE V.—Carcass Measurements—Series 2.

Ration.	Breed.	Number of Trials.	Number of Pigs.	Weight of Cured Side.	Weight of Shoulder as Percentage Weight of Whole Side.	Length of Side.	Depth of Back Fat at Shoulder.	Circumference of Ham.	Dressing Percentage of Pig.
Group 1.—*Dry Lot or Stye, no Separated Milk.*				lb.		in.	in.	in.	
Maize–Meatmeal.....	Tamworth x Large Black.....	1	15	54	30·6	29·0	2·1	23·4	83·5
	Tamworth x Large Black.....	2	12	50·1	30·5	28·8	2·2	23·2	83·8
	Average of two trials.....	—	—	52·0	30·5	28·9	2·1	23·3	83·6
Maize – Barley–Meatmeal	Tamworth x Large Black.....	1	12	50	30·2	28·8	1·9	23·4	84·1
Maize – Kaffircorn – Meatmeal	Tamworth x Large Black.....	1	12	52·3	30·4	28·7	2·3	22·9	84·1
Group 2.—*Full Fed on Kikuyu Pasture, with or without Separated Milk.*									
Maize – Meatmeal, Kikuyu Pasture	Large White x Large Black....	1	12	55	31·7	28·9	2·2	24·1	84·3
	Large White x Large Black....	2	12	57·9	31·3	29·0	2·2	24·6	83·9
	Large White x Large Black....	3	10	59	30·5	29·0	2·3	24·3	82·7
	Large White x Large Black....	4	20	55·8	29·5	28·2	2·2	24·3	85·0
	Large White x Large Black....	5	12	56·1	30·9	28·7	2·1	24·3	82·8
	Average of five trials.....	—	66	56·7	30·7	28·7	2·2	24·3	83·7
Maize – Kaffircorn – Meatmeal, Kikuyu Pasture	Large White x Large Black....	1	20	55·4	30·0	28·0	2·2	24·2	84·4
Group 3.—*Two-thirds Ration on Kikuyu or Lucerne Pasture, with or without Separated Milk.*									
Maize–Lucerne.....	Tamworth x Large Black.....	1	15	47·5	30·1	29·1	2·1	23·4	82·2
Maize–Meatmeal, Kikuyu	Large White x Large Black....	1	10	56·7	30·9	28·8	2·1	24·2	81·7

Percentage of Shoulder..... Shoulder cut from side between third and fourth rib.
Length of side..... Aitchbone to centre of the curve of the first rib.
Depth of back fat..... At thickest point on top of shoulder.

In comparing the maize-meatmeal lots in groups 1 and 2 a close similarity in the measurements of the two crosses is noted. Except for the slightly greater circumference of ham in the case of the Large White Cross (24.3 in. as compared with 23.3 in.) the measurements of the cured sides of the two crosses show little difference. The average depth of backfat at the shoulder 2.1 in. to 2.2 in. is over the standard of 1.75 in. generally set for " lean sizable " bacon sides of the weights given. Though these average figures do not represent the average measurements of the sides which were actually graded " lean sizable " in these experiments, it is probable that a proportion of the sides graded " lean sizable " at the Estcourt factory, though otherwise satisfactory, would have been criticised as " fat " on the London market.

Apart from the important advantage of a mostly white skin to the curer, in the Large White Cross, the two crosses produced sides of similar characteristics. In deciding the grade of carcass, the condition at marketing and the individuality of the pigs appear to be more important than the breed.

(2) *The Effect of Feed on the Type of Side produced.*

It is generally held that there is a close relation between the firmness of the fat and the amount of fat or oil in the ration. In this series half the maize therefore was replaced in certain rations by ground barley or by kaffircorn to determine whether the resultant reduction in the fat content of the grain ration would be reflected in the firmness and type of the sides produced.

From an inspection of Table IV it appears that the use of ground barley or kaffircorn has not increased significantly the proportion of " lean sizable " sides. These results do not confirm those of Series 1 where the replacement of a proportion of the maize by ground barley and pollard apparently resulted in an increase in the proportion of No. 1 " lean sizable " sides. The difference can be accounted for probably by the somewhat fatter conditions of the pigs in Series 2 at the time of marketing. This difference is noticeable in the barley-fed groups and accounts for some of the relative decrease in the proportion of " lean sizable " sides in Series 2. The results from the different rations within each series are comparable. They indicate, however, the importance of the condition of the pigs—as apart from weight—in interpreting the results of successive experiments of this nature.

Kaffircorn shows a distinct tendency to produce an overfat rather short side. This compactness was more striking on the hook than the figures in Table V indicate and caused unfavourable comments from the graders. This tendency is shown, however, in the average measurements recorded for the Tamworth cross, where the average length of side, depth of backfat and dressing percentage was 28.9 in., 2.1 in., and 83.6 per cent. respectively on the maize ration as compared with 28.7 in., 2.3 in., and 84.1 per cent. on the kaffircorn ration. The comparable figures for the Large White Cross are 28.7 in., 2.2 in., 83.7 per cent. and 28.0 in., 2.2 in., 84.4 per cent. respectively.

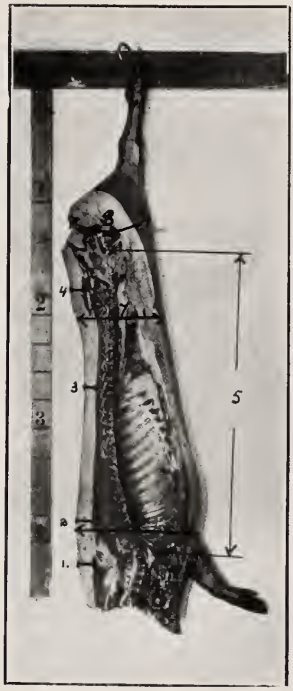


FIG. 4.

Measurements taken on the carcase.

1, 2, 3, 4. Depth of back
1, 2, 3, 4. Depth of backfat. 5. Length of carcase
6. Width at fore flank. 7. Width at hind flank.
8. Circumference of ham.

FIG. 5.

Well finished Large White—Large Black baconers.

TABLE VI.—The Refractive Indices of the Backfat—Series 2.

Ration	Breed.	No. of Pigs.	Firm.	Medium +	Medium −	Soft.	Refractive Average.
Group 1.—*Dry Lot or Stye, no Separated Milk.*							
Maize–Meatmeal.....	Tamworth x Large Black.....	18	11	7	0	0	1·4595
Maize–Barley–Meatmeal.....	Tamworth x Large Black.....	9	9	—	0	0	1·4591
Maize–Kaffircorn–Meatmeal.....	Tamworth x Large Black.....	12	6	6	0	0	1·4594
Group 2.—*Full Fed on Kikuyu Pasture, with or without Separated Milk.*							
Maize–Meatmeal.....	Large White x Large Black....	18	10	8	0	0	1·4595
Maize–Kaffircorn–Meatmeal.....	Large White x Large Black....	17	12	5	0	0	1·4593
Group 3.—*Two-thirds Ration on Lucerne Pasture.*							
Maize–Meatmeal.....	Tamworth x Large Black.....	15	3	4	4	6	1·4603

Refractive Index.

Scale of firmness: Firm..... 1·4595 and below.
Medium +.... 1·4596–1·4599.
Medium −.... 1·4600–1·4603.
Soft..... 1·4604 and above.

In these trials an increase in the allowance of separated milk to pigs on pasture has somewhat increased the proportion of No. 1 " lean sizable " sides, but the data are insufficient to determine whether this difference is significant. This is shown in group 2 of Table IV by comparing the results of trials 2 and 5 with those of trials 1, 3 and 4. The pigs in trials 2 and 5 received from 1 to 2 gals. of separated milk per day, those in trials 1, 3 and 4 received from no milk up to a maximum of $\frac{1}{2}$ gal. each irregularly per day. The percentage of No. 1 " lean sizable " sides in groups 2 and 5 is 33.3 per cent. as compared with 16.6 per cent. in the other three groups.

(3) *Effect of Feed on the Firmness of Fat.*

In the earlier trials the determination of firmness was based on the feel of the side. Owing to irregularities in the temperature of the sides at the time of the test the results proved unreliable and have had to be discarded. In the second series of trials the refractive index of the backfat was used as the measure of firmness of fat. All the available determinations are given in Table VI.

The scale of firmness adopted varies slightly from the one formulated by the workers on soft pork in the United States Department of Agriculture (Bul. 1407, 1926, and 1492, 1928) having been raised arbitrarily by .0002 points in each class with the object of securing a slightly greater degree of firmness in the various grades. Insufficient data have been obtained so far to say whether this arbitrary scale will work in practice.

The limited data so far available appear to show that the use of barley results in the hardening of the backfat, though, as pointed out in a previous section, it does not increase the proportion of No. 1 " lean sizable " sides. The average refractive index of the backfat in the barley fed pigs was 1.4591 as compared with 1.4595 in the comparable maize fed groups. All the barley fed pigs were graded hard as compared with 59 per cent. of the maize fed pigs.

From results available it cannot be said whether kaffircorn has a hardening effect or not.

The comparative softness of the fat of the pigs on the lucerne pasture is partly due to their being relatively less " finished " than the other groups at the time of marketing.

GENERAL CONCLUSIONS.

Breed of Pig.

In these experiments the Tamworth-Large Black Cross and the Large White-Large Black Cross have proved superior to the Middle White-Large Black Cross for the production of " lean sizable " bacon.

The Large White-Large Black Cross has the advantage of a white skin, better quality of meat and an absence of seedy cut over the Tamworth-Large Black Cross, but from a feeding standpoint the two crosses have proved equally satisfactory.

Maize Ration.

The maize-meatmeal ration supplemented, where possible, by separated milk and pasture has proved the most efficient in these experiments.

The addition of ground barley, pollard or kaffircorn to this ration did not increase the economical rate of gain.

The addition of barley meal and pollard increased the proportion of sides graded No. 1 " lean sizable " in the first series of trials, but in the second, where conditions in regard to grading were better

controlled, the use of these feeds did not increase the proportion of No. 1 " lean sizable " sides.

In a ration of maize meal 45 parts, barley meal 45 parts, and meatmeal 10 parts, barley meal has a hardening effect on the fat when compared with a ration of maize meal 90 parts and meatmeal 10 parts. Under present conditions the use of barley, however, is not justified from an economic standpoint as this improvement is not reflected in the selling price.

A ration of maize 45 parts, kaffircorn 45 parts, and meatmeal 10 parts produced carcasses which, on the limited data available, were intermediate in hardness between the maize meal and ground barley rations. On account of their overfat conditions these carcasses, however, were undesirable for the production of Wiltshire bacon.

PROPOSALS FOR FUTURE INVESTIGATIONS.

Trials are in progress to confirm, or refute, the results obtained in Series 2. All the work summarised in this report seems to indicate, however, that, under local conditions, it is not practicable to modify the type of pig by changes in the rations. The general type of pig in South Africa at present lacks length, is relatively heavy in the shoulder and carries too deep a layer of back fat. If improvements in these respects are to be effected, it will probably be by methods of selective breeding rather than by changes in the feeding practices.

In countries where the feeding practices are more standardised than in the Union, progress is now being effected through some system of " Pig Recording " in which the actual data on the performance of the progeny of a sow is used as a basis of selection of the breeding stock. A scheme with this end in view has been outlined by Davidson and Duckham (1929) in a recent report on the East Anglian Pig Recording Scheme.

It is proposed to commence similar " type-testing " work at the Experiment Station at Cedara and at the Veterinary Research Station near Ermelo. It is hoped thereby, first to determine and then to develop, the most satisfactory type or cross of pig, taking into consideration both local conditions and the export market.

SUMMARY.

1. Data have been given on 329 pigs which were fed various modifications of a standard maize-meatmeal ration to determine the effect of the modifications on the grade of bacon produced.

2. The general lack of effect of these changes in the ration is noted.

3. Suggestions are made as to the future course of the work, with special reference to the establishment of two " type-testing " stations.

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Section X.

Miscellaneous.

S. D. Rossouw...	A Short Study of the Isotonicity of Sheep Blood.
A. S. Canham ...	Blood of Cattle.
F. Brauns	The Water Consumption of Sheep in a Nutrition Experiment at Onderstepoort.
H. H. Curson and N. T. v. d. Linde	Anatomical Studies No. 14. Anomalies of a Cross-bred Ram.
H. H. Curson ...	Anatomical Studies No. 15. Accessory Spleens in a Horse.
H. H. Curson ...	Anatomical Studies No. 16. On two cases of Free-Martinism.
H. H. Curson and A. D. Thomas	Anatomical Studies Nos. 17. Epidermoid Cysts in Domesticated Animals.
H. H. Curson ...	Anatomical Studies No. 18. Dental Anomalies in the Ox and Horse.

A Short Study of the Isotonicity of Sheep Blood.

By S. D. ROSSOUW, B.Sc., Research Officer, Onderstepoort.

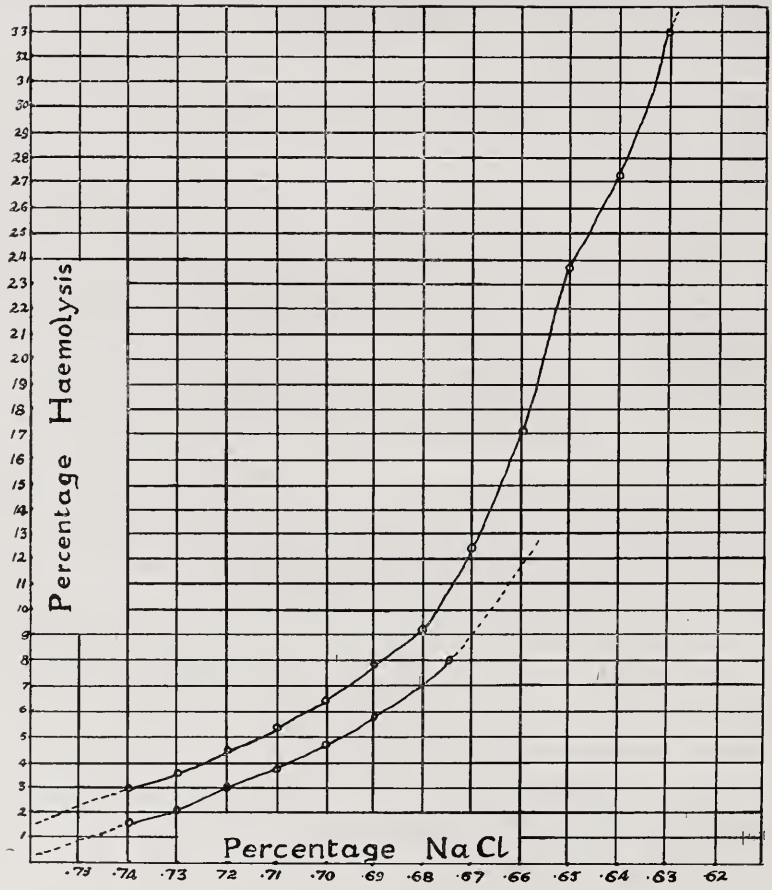
In this study an attempt was made to find out what constitutes isotonic saline for the blood of sheep. For the work in progress here at Onderstepoort it is necessary to have more exact data on this question of isotonicity of sheep blood than one is able to obtain from the available literature. Hence a few short experiments were carried out.

Strictly speaking an isotonic solution is also an isosmotic solution, for a particular blood corpuscle, i.e. a solution which will have the same osmotic pressure as the contents of the blood cell. In general, we use isotonicity in a much broader sense, and regard it as a solution of such concentration that it does not cause the cell membrane to rupture (haemolysis of the cell) nor does it cause undue crenation due to its higher salt concentration. An example of the popular application of isotonicity is the following: Most of the corpuscles of a certain blood will rupture in contact with a sodium chloride solution of concentration, 0.72 per cent. and will not be unduly crenated at a concentration of 1.10 per cent. The solution of sodium chloride is then regarded as isotonic along the whole range of 0.72 per cent. to 1.10 per cent. and for practical purposes .9 per cent (say) of sodium chloride solution can be used.

The above may be perfectly correct when dealing with a single or a certain type of erythrocyte, but in considering collectively the cells of, say 1 c.c. of blood, the problem becomes more difficult.

From the curves, it will be noticed, that haemolysis may start at, 0.76 per cent. NaCl and only reach completion, i.e. 100 per cent. haemolysis, at about 0.58 per cent. NaCl. Thus some cells may haemolise at 0.76 per cent., whereas the more resistant ones will do so only at 0.58 per cent. One must not conclude from the above that any concentration of sodium chloride above 0.76 per cent., i.e. before haemolysis begins, may be used. For, naturally, in a 0.95 per cent. saline, no haemolysis will occur, but marked crenation of the cells will be noticed. Hence it is obvious that a solution of sodium chloride which approaches isotonicity most closely is one just about 0.76 per cent., say 0.8 per cent. It is not necessary to establish the point of maximum resistance (Abderhalden) but only that of minimum resistance, i.e. when haemolysis begins.

Much work has been done on the resistance of red blood cells, by Snapper, Brinkman and Van Dam, and Hamburger. Not only did they improve the technique for determining the true point of


Percentage Haemolysis
Percentage NaCl
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
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minimum resistance accurately, but also threw much light on the chemical and physical nature of the erythocyte. Most of the work was done on the blood of human beings, rabbits, horses, bovines, etc., but as far as could be ascertained, no work of this nature has been done on the blood of sheep. From the results given by Hamburger the erythrocytes of horse blood are least resistant, viz. .62 per cent. NaCl as an average.

It was decided to use blood directly from the vein, as Hamburger suggests, although the procedure followed here is somewhat different. Anticoagulants were ruled out as their addition is bound to cause changes in the physical properties of the cell, and hence probably faulty results. Defibrinated blood might be used but defibrination is apt to result in slightly tinged serum, which will interfere with the determination.

Brinkman and Van Dam used " equilibrierter " physiological saline and then determined the point of minimum resistance or the haemolysis curve by decreasing only the sodium chloride content of the physiological saline and keeping the other constituents constant.

The method followed here will not give the same absolute results as that of Brinkman and Van Dam, but for comparative purposes it is quite accurate enough and, furthermore, constitutes a quick method for routine use.

The haemolysis curves were obtained in the following way. A number of solutions of sodium chloride, from 0.60 per cent. to 0.74 per cent. and differing by 0.01 per cent. was made up from a stock solution of 0.85 per cent. 20 c.c. of each of these solutions are pipetted into 25 c.c. centrifuge tubes. One tube contains 20 c.c. of distilled water. The sheep are bled from the jugular vein, and before the blood can coagulate one c.c. is pipetted into each of the centrifuge tubes and well mixed. This part of the work requires great care and dexterity. The tubes are allowed to stand for about 15 minutes and then centrifuged at a speed of about 3,000 revolutions per minute for 10 minutes. As was verified by the author, it makes little or no difference whether the tubes stand for 5 minutes or 2 hours. It is, however, important that the tubes be centrifuged at a high speed because, if not, the leucocytes, which have a lower rate of sedimentation, will cause turbidity in the supernatant fluid.

The contents of the tube containing distilled water and blood represent 100 per cent. haemolysis. From this solution various standards are made up by dilution. The supernatant liquid in the various tubes is matched in a colorimeter against a suitable standard and thus the degree of haemolysis determined at different concentrations of NaCl. By this method it is possible to determine haemolysis down to 2 per cent., which in the above concentration gives almost a clear solution.

As sodium chloride solution, whether isotonic or not, disturbs the chemical and physical condition of the cell it will not give the true value for haemolysis as was found by Snapper, Brinkman and others, but it gives a figure for minimum resistance slightly lower than the

correct one; i.e. will only show haemolysis at a higher saline concentration than is actually the case. At the point of minimum resistance the concentration of sodium chloride solution will not differ much from the true value.

This variation is due to the calcium and lipoid contents of the cell. These tend to be fairly constant for sheep blood.

The two haemolysis curves given are typical for the blood of sheep. The lower portions are similar in shape and can be used for purposes of obtaining the degree of haemolysis at definite concentration of sodium chloride. For instance, if a certain blood sample with NaCl 0.70 per cent. shows 6.5 per cent. haemolysis, then, from the curves it would show 3 per cent. haemolysis at 0.74 per cent. NaCl. On verification the particular blood sample gave the results deduced from the curves.

Thus for routine purposes the matter is greatly simplified. The tubes are taken in pairs, one tube containing 20 c.c. of a definite saline solution, say 0.74 per cent. of NaCl and the other 20 c.c. of distilled water. A number of pairs like this is arranged and the animals are bled; one c.c. of blood from each is pipetted into each tube of a pair. The tubes are centrifuged and the degree of haemolysis determined colorimetrically.

From the graph the NaCl concentration producing a definite haemolysis, such as 3 per cent. say, is read off. Three per cent. was found to be the lowest practical margin to work at. Should the value differ greatly from 3 per cent. then it may be necessary to make a second determination.

In all cases up to 10 per cent., the deduced value from the graph was found to agree with the value as determined. The following are some figures found for normal sheep at this Institution:—

Sheep No.	*Percentage NaCl producing 3 Per cent. Haemolysis.*	*Bled two days afterwards.*
25513	0·78	0·76
24188	0·73	0·73
24195	0·74	0·72
24193	0·72	—
24196	0·74	—
25746	0·75	—
25701	0·79	—
25698	0·75	—
25737	0·78	—
25722	0·79	—
25803	0·80	—
25795	0·79	—
25715	0·81	—
25645	0·73	—
AVERAGE	0·76	

When these extraordinary high saline values as compared with other animals were obtained (low resistance for blood cells) for the blood of normal sheep, some comparative figures were determined for normal bovine blood.

Bovine No.	*Percentage NaCl producing 3 Per cent. Haemolysis.*
2885	0·62
3502	0·68
3535	0·64
3524	0·67
3544	0·65
AVERAGE	0·65

This average figure of 0.65 agrees fairly well with the value of 0.60 for bovine blood given in Abderhalden. Some workers use 0.80 per cent. NaCl which they regard as isotonic for blood in general. That such is not the case is clearly shown from the results for the blood of sheep, 0.85 per cent will be a much better concentration, and to be definitely within the safe zone it will be better to use 0.90 per cent. solution for the blood of sheep.

In an exceptional case the writer found an animal, No. 25750, which showed 3 per cent. haemolysis at a concentration of 0.93 per cent. NaCl. This was subsequently found to be an animal injected with " bluetongue " vaccine a few days previously.

When more extensive data for normal sheep blood have been obtained the study of pathological blood will be commenced.

SUMMARY.

1. In this paper it has been shown that the erythrocytes of normal sheep blood are much less resistant to hypotonic sodium chloride solutions than any other kind of blood dealt with so far.

2. 0.9 per cent. NaCl is the most practical isotonic solution for the blood of sheep.

The author gratefully acknowledges his indebtedness to Professor A. I. Malan for his advice and suggestions during the investigation.

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Blood of Cattle.

By A. S. CANHAM, F.R.C.V.S., Government Veterinary Officer, Bloemfontein.

This work was commenced at the School of Agriculture, Potchefstroom, West Transvaal, on the suggestion of Sir Arnold Theiler, Director of Veterinary Research and Education, Union of South Africa in 1924 and submitted to the Royal College of Veterinary Surgeons in 1926 as a thesis for the F.R.C.V.S. examination by the writer. It was written as a corollary to the work of Neser on Blood of Equines (1923). This last mentioned work is especially noteworthy for the methods of technique and the adaptation of them to conditions in the field. Most haematological studies on the blood of cattle have been carried out in countries other than the Union and naturally the conditions found in these different parts vary considerably. Consequently this work could well be entitled "Blood of South African Cattle." Only healthy, normal cattle have been worked on.

With this in view, the work has been divided into different parts:—

1. Technique employed.
2. General observations on the blood of bovines.
3. Observations on the influence of age.
4. Influence of exercise.
5. Influence of pregnancy and parturition.
6. Influence of oestrum.
7. Influence of altitude.
8. Influence of lactation.
9. Leucocytes of cattle—description, number, differential counts and general observations.

1. TECHNIQUE.

The technique used in this work is practically identical with that emplyed by Neser in his work on the Blood of Equines with two small exceptions. The first modification was in connection with the tubes for precipitation of the red blood cells—instead of cutting glass tubing, very small test tubes of uniform bore were heated and the ends were flattened, before they were graduated and instead of using .5 c.c. of liquid for Neser's mark L_1 .75 c.c. of liquid was used and finally for mark L_2 1.5 c.c. of liquid was added. The second modification was in connection with the actual amount of blood tapped off for the blood examination. 25 c.c. of blood was taken from each animal as against 10 c.c. of Neser. All glassware was cleaned with a solution consisting of equal parts of absolute alcohol and acetone.

Abstraction of Blood from the Jugular Vein.

In all cases blood was taken from the jugular vein by means of a short hypodermic needle of large calibre previously sterilized. It is more difficult to carry out this small operation in some breeds (e.g. Africander and Africander Crosses) than in other breeds. This is on account of the thickness of the skin, the folds of the flesh and their wildness.

Precipitation.

For this purpose all samples were precipitated by means of a water turbine centrifuge. All tubes were tested against one another prior to their being used for this work. Two tubes were used for each sample of blood so as to check one against the other. The centrifuge was allowed to run until an end result was obtained and then for a further hour to ensure that complete precipitation had taken place.

Blood Counts.

For the first part of this work the counting chamber used was Bürker's, but for the major portion a later and improved pattern of Bürker's was utilized.

Staining of Smears for Differential Counts of Leucocytes.

The procedure as advised by Pappenheim and carried out by Neser was to an extent adopted in this work. It was found later, however, that even better results perhaps were obtained by staining with 10 per cent. Permanganate Blue solution and 1 per cent. Eosin solution (Stèvenel, 1921). This method of staining made the comparison of the differences in the structure of the nuclei of the lymphocyte and monocyte easier.

2. GENERAL OBSERVATIONS ON THE BLOOD OF BOVINES.

No work was done on the haemoglobin content of bovine blood nor was the specific gravity inquired into.

Colour of the Blood and Serum.

It was frequently noticed that the blood of some animals was much lighter in colour than the blood of others. This variation was very marked at times when the bloods of young and old animals were compared. In centrifuging these light coloured samples it was found that the serum was not the usual yellowish colour but resembled more a slightly, cloudy water—rather a milk and water appearance. It was marked in blood samples taken from calves aged from 4 hours to 3 days, especially if they had been kept in a pen from the time of birth. The serum of dark coloured blood was found to be of a distinct yellowish colour. This was the case in oxen that were worked hard and also in high conditioned cows in milk. The serum of many scrub animals is often very pale in spite of a normal red cell count —this is especially the case in young cows living under drought conditions and having to nourish, perhaps, a calf for a long time.

Shape of the Erythrocytes.

The conclusions arrived at in this paper were that under different conditions the shape of the red cells varied from biconcave discs to bell-shaped masses of protoplasm.

In a number of young male animals, the red cells were between 3-4μ in diameter. However, very large erythrocytes about 7μ in diameter were observed in adult bovines. Cells having a diameter of 6μ were fairly frequently seen. The range of size consequently lies between 3μ to 7μ. The greatest variations in the size of the red cells were seen in the blood of young animals while the measurements of red cells in cows, bulls and oxen were more nearly constant.

Blood Platelets.

In all smears examined, these bodies were found, sometimes thickly covering the smears or only in small numbers. In the majority of cases they were found clumped together but now and again appeared as single cells lying between the red blood cells. They resembled oval bodies when fairly large, and round when of small size. In smears stained by May-Grünewald and Giemsa they show a number of granules, some small and some large. No counts were made of these bodies.

Relation between Volume of Red Corpuscles and their Number.

Following Neser's method the ratio of volume to their number was determined. The results are seen in the table below:—

Class of Animals.	Sex.	Age.	RP/RC.	Number of Animals Concerned.
Bovine.....	M.	1 day to about 12 months	3·8	72
,, ...	F.	,, ,, ,,	3·8	87
,, ...	M.	14 months to about 36 months	4·5	13
,, ...	F.	,, ,, ,,	4·3	14
,, ...	M.	Adult.....	4·7	43
,, ...	F.	,, ...	4·7	60

It was noticed that in young animals, both male and female, the ratio was low and it remained low up to the age of 13 months. From 14 months to about 3 years the ratio was increased, while in adult animals it was still higher. The average ratio of cattle of all ages works out to approximately 4.4.

3. OBSERVATION ON INFLUENCE OF AGE.

As a more or less preliminary study the influence of age on the red cell counts was considered, bearing in mind, however, the results of Neser which showed that exercise affected the red cell count more than age. The influence of exercise was borne out to an extent when one considered the counts of young animals kept in a pen for some days as compared with the counts of young animals allowed to run out in the open very soon after being born.

Bovine.	Sex.	Age.	Where Kept.	R.C.	R.P.	R.P./R.C.
No. 1....	M.	1 day.....	In pen.....	7,860,000	34·3	4·3
No. 1....	M.	3 days....	In pen.....	7,560,000	35	4·6
No. 1....	M.	21 days...	In veld for 11 days..	8,360,000	36	4·3
No. 150..	M.	1 day.....	In pen.....	6,440,000	23	3·6
No. 150..	M.	9 days....	In pen.....	6,480,000	24	3·7
No. 150..	M.	22 days...	In veld for 12 days..	8,120,000	31	3·8
No. 159..	F.	2 weeks...	In veld for 4 days..	8,930,000	34	3·8
No. 159..	F.	3 weeks...	In veld for 11 days..	10,880,000	44	4·0
No. 161..	F.	5 days....	In pen.....	9,060,000	40	4·4
No. 161..	F.	1 month...	In veld for 20 days..	10,330,000	39	3·9

If such young animals are confined in pens and not allowed exercise their red cell counts start to drop temporarily up to the time they are allowed out when once more their counts rise for a time.

Störch, quoted by Burnett (1908), gives a list of figures which refers to red cell counts of animals of different ages, viz:—

Young cattle ... ... 7,055,000 Red cells per c.mm.
Calves ... ... 8,523,000 Red cells per c.mm.

From figures obtained in this work (see Table H.3) one will observe that the red cell counts appear higher. A few examples are given:—

Class of Animals.	Age.	Sex.	R.C.
Bovine No. 5.....	8 days.....	M.	10,580,000
Bovine No. 151.....	2 months.....	M.	9,820,000
Bovine No. 64.....	6 months.....	M.	9,680,000
Bovine No. 6.....	3 days.....	F.	10,030,000
Bovine No. 149.....	2 months.....	F.	9,500,000
Bovine No. 147.....	6 months.....	F.	10,900,000

Furthermore calves of both sexes, one day old, often show a ratio of percentage volume of red cells to red cell count of at least 4.

Bovine.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
No. 1.....	M.	1 day.....	7,860,000	34	4·3
No. 5.....	F.	1 day.....	10,820,000	44	4·0
No. 151.....	M.	1 day.....	11,480,000	51	4·4
No. 150.....	M.	1 day.....	6,440,000	23	3·6

By the time these animals reach the age of from 2 to 3 months this ratio has fallen to about 3.8 and remains at this level for some time.

Bovine.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
No. 161.....	F.	1 month.....	10,330,000	39	3·9
No. 149.....	F.	2 months....	9,500,000	36	3·7
No. 1, Cedara.....	F.	2 months....	11,840,000	46	3·8
No. 151.....	M.	2 months....	8,820,000	37	3·7
No. 149.....	M.	2 months....	10,040 000	35	3·5
No. 107.....	M.	3 months....	8,400,000	32	3·7

Some calves when born show low initial red cell counts. These also, however, show early the influence of exercise, increasing often by nearly 1,000,000 or more in a short time.

Bovine.	Sex.	Age.	R.C.
No. 1.....	M.	1 day.....	7,860,000
No. 1.....	M.	21 days.....	8,360,000
No. 150.....	M.	1 day.....	6,440,000
No. 150.....	M.	22 days.....	8,120,000
No. 161.....	F.	5 days.....	9,060,000
No. 161.....	F.	1 month.....	10,330,000

After working on a large number of young male and female bovines aged from 1 day to 13 months it was shown that the average for them was in the region of: —

Class of Animals.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
Bovines.....	M.	1 day to 13 months.	9,000,000	34 (see table 1)	3·8
Bovines.....	F.	1 day to 13 months.	9,300,000	35 (see table 3)	3·8

There is a gradual drop in the counts of red cells in calves from the usually initial high counts as the animal gets older, e.g.: —

Bovine.	Sex.	Age.	R.C.
No. 64.....	M.	5 months....	10,420,000
No. 64.....	M.	6 months....	9,680,000
No. 64.....	M.	7 months....	8,300,000
No. 64.....	M.	$7\frac{3}{4}$ months...	8,700,000
No. 64.....	M.	8 months....	8,200,000
No. 147.....	M.	$7\frac{1}{2}$ months...	9,000,000
No. 147.....	M.	11 months...	8,580,000
No. 9.....	M.	5 months....	9,400,000
No. 9.....	M.	6 months....	9,400,000
No. 9.....	M.	7 months....	8,800,000
No. 9.....	M.	8 months....	7,300,000

Similarly from the work on a number of male and female bovines aged from about 14 months to about 3 years it was shown that the average for them was in the region of:—

Class of Animals.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
Bovines.....	M.	14 months to 3 years	7,600,000	35 (see table 2)	4·5
Bovines.....	F.	14 months to 3 years	7,300,000	34 (see table 4)	4·3

When we come to the consideration of the red cell counts of adult cows we find that they differ slightly under different conditions, e.g.:—

Bovine.	Sex.	Age.	Conditions.	R.C.
No. 116.....	F.	Adult...	In calf for $2\frac{1}{2}$ months.....	6,880,000
No. 116.....	F.	Adult...	In calf for $3\frac{1}{2}$ months.....	6,460,000
No. 116.....	F.	Adult...	In calf for 5 months.....	6,660,000
No. 126.....	F.	Adult...	1 month after calving.....	5,700,000
No. 126.....	F.	Adult...	$1\frac{1}{2}$ months after calving.....	5,600,000

Bovine.	Sex.	Age.	Conditions.	R.C.
No. 20.....	F.	Old Adult.	12 years and 2 months.....	5,890,000
No. 20.....	F.	Old Adult.	12 years and 4 months.....	7,080,000
No. 4 C.....	F.	Old Adult.	14 years and 8 months.....	6,840,000
No. 4 C.....	F.	Old Adult.	14 years and 10 months.....	6,980,000
No. 9.....	F.	Old Adult.	14 years and 9 months.....	5,500,000
No. 7.....	F.	Old Adult.	17 years and 9 months.....	5,270,000

It was found that the average for cows under all conditions was in the region of:—

Class of Animals.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
Bovines.....	F.	Adult.....	6,660,000	(see tables 5, 6, 7, 8, 9).	4·7

Conclusions.

(*a*)

Class of Animals.	Sex.	Age.	R.C.	R.P.	R.P./R.C.
Bovines.....	M.	Birth to 14 months.....	9,000,000	34	3·8
Bovines.....	F.	Birth to 14 months.....	9,300,000	35	3·8
Bovines.....	M.	14 months to 3 years....	7,600,000	35	4·5
Bovines.....	F.	14 months to 3 years....	7,300,000	34	4·3
Bovines.....	F.	Adult.....	6,660,000	31·9	4·7

(*b*) The red cell counts of male calves is lower than that of female calves, the volume percentage is also lower but the ratio between red cell counts and percentage volume of cells is the same in young males as in young females.

(*c*) The red cell count in old female bovines tends to diminish with age.

4. INFLUENCE OF EXERCISE.

The "manner of life" has always been kept in view in this work and it appears that the influence of this factor is as marked with cattle as with horses. The divisions of stabled animals, slow-working animals and fast-working animals cannot be used in connection with bovines, but one might divide the animals into:—

(*a*) Young bovines confined to a pen.
(*b*) Young bovines allowed to run and then confined to a pen.
(*c*) Dairy and veld cows.
(*d*) Oxen—hard working.

Burnett (1908) states that "a considerable increase in the number of leucocytes has been found after severe muscular exertion." Larrabee, according to Burnett, examined the blood of four contestants in a 25 mile running race before and immediately after the finish of the race and he found a leucocytosis of 14,000 to 22,200. In the article by Dufton (1917) it is shown that in rabbits exposed to large amounts of carbon di-oxide (3-4 per cent.) there is an increase in the number of red cells for the time of exposure; this increase drops after the rabbits' removal to the ordinary atmosphere. She states that Kolls and Loevenhart (1914) showed there was an increase of corpuscles and haemoglobin in animals "subjected to oxygen want." Her conclusions were "any factor, such as violent exercise which produces a temporary rise in the concentration of carbonic acid in the blood will produce a corresponding formation of fresh corpuscles." Hill and Lupton (1922) showed there was usually an oxygen deficiency or "want" during violent exercise and in the light of Kolls and Loevenhart's work there must have been an increase in the red corpuscles and according to Larrabee an increase in the white cells. Neser's results in Table III, page 542 *re* Horse 11144 showed that after hard, fast exercise both red and white cell counts were increased, but after slow exercise the white cell count only was raised. All these results of increase in counts are only transient and one will attempt to show that with continuous work, which is a form of steady, prolonged exercise, the red cell count is raised.

(*a*) Young Bovines Confined to a Pen Immediately after Birth.

These animals were Fries calves—male and female—and, as is the custom here, were kept in a pen from two days to a week or more. They were consequently permitted only a small amount of restricted movement and were hand-fed two or three times during the day. As pointed out in the earlier part of this work, their red cell count if taken a few hours after birth or within the first day or two of life is high in the majority of cases. Their count gradually drops as long as they are kept in a confined space.

Class of Animals.	Sex.	Age.	R.C.	Conditions.
Bovine No. 6.....	F.	1 day.....	10,960,000	Kept in pen.
Bovine No. 6.....	F.	3 days.....	10,030,000	Kept in pen.
Bovine No. 6.....	F.	7 days.....	9,680,000	Kept in pen.
Bovine No. 6.....	F.	12 days.....	9,265,000	Kept in pen.
Bovine No. 7.....	F.	1 day.....	9,964,000	Kept in pen.
Bovine No. 7.....	F.	3 days.....	9,504,000	Kept in pen.
Bovine No. 7.....	F.	7 days.....	9,336,000	Kept in pen.
Bovine No. 7.....	F.	10 days.....	9,448,000	Kept in pen.
Bovine No. 7.....	F.	15 days.....	9,176,000	Kept in pen.
Bovine No. 1.....	M.	1 day.....	7,860,000	Kept in pen.
Bovine No. 1.....	M.	3 days.....	7,560,000	Kept in pen.

As soon as they are allowed to go out, they run and jump about and race up and down the fields—the result is an increased red cell count.

Class of Animals.	Sex.	Age.	Conditions.	R.C.
Bovine No. 6.....	F.	12 days.	Confined in pen.....	9,260,000
Bovine No. 6.....	F.	21 days.	9 days exercise.....	11,392,000
Bovine No. 6.....	F.	30 days.	18 days exercise.....	11,296,000
Bovine No. 161.....	F..	5 days..	Confined in pen.....	9,060,000
Bovine No. 161.....	F.	30 days.	25 days exercise.....	10,330,000
Bovine No. 1.....	M.	3 days..	Confined in pen.....	7,560,000
Bovine No. 1.....	M.	21 days.	18 days exercise.....	8,360,000
Bovine No. 150.....	M.	9 days..	Confined in pen.....	6,480,000
Bovine No. 150.....	M.	22 days.	13 days exercise.....	8,120,000

After about a month's exercise, perhaps a little longer, the counts fluctuate but always with a downward trend until in the case of bull calves by about the 9th month the counts reach a fairly constant level. Thereafter the number continues to decrease till about the 19th to 24th month. By the time they reach the 30th and 36th month their average count is between 6,700,000 to 7,600,000. From the time they are first allowed out their energy, or rather their inclination to exercise, decreases with age and by the time they reach the 30th to 36th month, they are content to walk about and graze quietly or lie and ruminate.

In the case of female calves the red cell counts do not appear to drop as quickly as those of males, the most marked difference is at about the age of 14 months when their average count is in the vicinity of 9,3000,000 red cells per c.mm. as compared with 9,000,000 red cells per c.mm. of the male. After that time their counts fall rapidly and from 14 months to 2 years or more their average shows 7,300,000 red cells per c.mm. as compared with 7,600,000 red cells per c.mm. of the male.

(*b*) Young Bovines allowed to run and then confined in a Pen.

A number of young bovines are sold every year from this Institution and about 3 months prior to the sale they are brought in and

prepared for the sale. It is from these animals that counts were obtained for the above heading: —

Class of Animals.	Sex.	Age.	Conditions under which Kept.	R.C.
Bovine No. 130.	M.	11 months.	Running in veld whole 11 months	9,120,000
Bovine No. 139.	M.	11 months.	Under same conditions as No. 130 for 8 months, in pen for 3 months	7,912,000
Bovine No. 135.	M.	12 months.	Running in veld for whole 12 months	10,090,000
Bovine No. 134.	M.	12 months.	Under same conditions as No. 135 for 9 months, in pen for 3 months	7,192,000

As these animals were sold and were lost to us, we were unable to obtain further blood counts. From counts of animals allowed to run the whole time one would surmise that their counts (139-134) would perhaps be likely to rise once more to nearer the average.

(*c*) Dairy and Veld Cows.

The counts were made in the majority of cases from pedigree Frieslands and a few from pedigree Ayrshires. These are high producing animals and many are in the advanced Registry. The stockman takes a pride in them and they are taken in the early moring to where there is plenty of good grazing and plenty of shade. They are brought back quietly in the late afternoon and milked. They are dipped in an arsenical dip once a week in the summer and never in the winter. The result is that they have a very small amount of exercise and so could perhaps be classed on the same plane as stabled horses.

Besides the factor of exercise one has also to consider the factors of lactation and pregnancy in connection with dairy cows. This, undoubtedly, has some influence on the red and white cell counts.

It will be found that Störch quoted by Burnett gives the average count of dairy cows as 5,473,000 red cells per c.mm. and Stöltzing quoted by Burnett as 5,073,000 red cells per c.mm. The counts obtained in this work are much higher, being for dairy cows under all conditions 6,660,000 red cells per c.mm. with a percentage volume of 31.9 for dairy cows under all conditions. Under conditions possibly prevailing in Germany they are kept in stables for a considerable part of the year and so the amount of exercise they obtain is very much less than that enjoyed by South African animals which are out practically every day of the year. This is offered as a possible explanation of the difference in the counts.

If, however, this should be the case, one would expect veld cows to have even higher counts than dairy cows. This, in the majority of cases, does not appear to be so, and probably other factors, e.g. class of animal, food and management, beside that of exercise, influence the red counts of veld cows. One can appreciate the likely effects, on non-descript cows, of drought conditions when they have often the additional burden of having to nourish a calf for months;

especially marked are these when they are entirely dependent, for sustenance, on the food they graze. A few examples may here be quoted: —

Class of Animals.	Sex.	R.C.	R.P.	W.C.
Dairy Bovine No. 116.....	F.	6,880,000	33	7,000
Veld Bovine No. 8.....	F.	5,900,000	29	11,400
Dairy Bovine No. 112.....	F.	7,200,000	34	8,000
Veld Bovine No. 9.....	F.	5,800,000	30	13,900
Dairy Bovine No. 79.....	F.	6,900,000	29	10,400
Veld Bovine No. 17.....	F.	6,270,000	28	11,200
			(see tables 6 and 8)	

In a minority of cases in the comparison of dairy cows and veld cows receiving a supplementary ration one will find the factor of exercise well brought out. Veld cows that are the progeny of a good sire and a " scrub " dam and have been well looked after may have their red cell counts higher than those of dairy cows.

Class of Animals.	Sex.	R.C.	R.P.	W.C.
Dairy Cow No. 112.....	F.	7,200,000	34	8,200
Veld Cow No. 1.....	F.	7,800,000	34	11,000
Dairy Cow No. 82.....	F.	6,400,000	26	6,400
Veld Cow No. 7.....	F.	7,100,000	32	10,700

It will be noted that the white cell count of veld animals whether well-cared for or not is greater than the white cell count of dairy cows.

The average figure for veld cows is in the vicinity of: —

Class of Animals.	Sex.	R.C.	R.P.	R.P. R.C.
Veld Cows.....	F.	6,100,000	29 (see table 8).	47

(*d*) Oxen.

The blood counts of oxen show the real effect of much exercise and especially hard work. They cannot be said to do fast work but they do hard, slow and continuous work and in the majority of cases all the food they get is what they graze—now and again they are given a little extra hay.

Counts were made on six young oxen that had only just been broken in to the yoke and the remaining counts were of oxen doing hard prolonged work. All these oxen were Africander crosses

and were in good condition. The average red cell count of oxen is higher than that of dairy cows: —

Class of Animals.	Condition.	Sex.	Age.	R.C.	R.P.	W.C.
Bovine No. 2...	Just trained.....	M.	Young...	6,910,000	33	11,500
Bovine No. 3...	Just trained.....	M.	Young...	6,790,000	33	9,700
Bovine No. 4...	Just trained.....	M.	Young...	6,940,000	31	8,100
Bovine No. 16..	Just trained.....	M.	Young...	7,210,000	34	8,300
Bovine No. 21..	Just trained.....	M.	Young...	6,960,000	33	11,200
Bovine No. 84..	Just trained.....	M.	Young...	6,290,000	30	7,500
Bovine No. 49..	Worked for long time	M.	Adult...	8,180,000	35	6,400
Bovine No. 17..	Worked for long time	M.	Adult...	9,300,000	43	11,400
Bovine No. 81..	Worked for long time	M.	Adult...	8,480,000	41	7,400

(see table 10)

As a result of the examination of a number of oxen it was found that the average counts were in the vicinity of: —

Class of Animal.	R.C.	R.P.	W.C.
Oxen.....	7,480,000	35	8,700

(see table 10)

Conclusions.

1. Similar to Neser one found that among cattle, as among horses, the mode of life is a very important factor that influences the red cell counts, white cell counts and the percentage volume of red cells.

2. Young calves confined to a pen immediately after birth show a gradually declining red cell count, but after they are allowed exercise the red cell count, white cell count and percentage volume increase.

3. Young bovines that are allowed exercise and then are confined to a box show a gradual decline in red cell counts.

4. High class dairy cows, well-looked after, show a more or less constant red cell count but it appears that pregnancy and lactation also influence the red cell counts.

5. Veld animals, typical scrub cattle, in spite of much exercise show usually a lower red cell count, but a higher white cell count than dairy cattle—this is probably due to the very adverse conditions under which they live. Good grade cows reared under good veld conditions usually show a higher red cell count than dairy cows.

6. Oxen show the highest red cell counts of all adult cattle, and the influence of work is apparently to bring about a higher red cell count.

5. INFLUENCE OF PREGNANCY AND PARTURITION.

(*a*) Influence of Pregnancy.

Burnett (1908) states that "normal pregnancy seems not to affect the number of red corpuscles as a rule" and quotes Cohnstein who, in connection with sheep, states he found an average of 9,742,000 red cells in 7 pregnant sheep and an average of 12,090,000 red cells in 5 non-pregnant sheep. Thompson (1904) gives the result of the blood examination of 12 pregnant women at different stages of gestation as follows:—"Moderate increase in red corpuscles rather early in pregnancy, remaining subnormal throughout the middle months and rising again to normal at the termination of pregnancy—not, however, in all cases." Neser states for the pregnant mare:—"The influence of work upon the erythrocyte count is so marked in the horse that unless this factor was carefully controlled for a large number of mares in foal and not in foal it would be very difficult to determine the influence of pregnancy."

After attempting to work on the effect of pregnancy on the red cell count, it is brought home to one how true this statement is. In a high-class milking herd it is most difficult to arrange to keep controls. One would almost say for such cows the pregnant stage is the normal one, for the reason that soon after parturition they have conceived once more. The result is that one is more or less compelled to obtain an average for all cows, e.g. all dairy or veld cows under every condition and then use this count as a basis of comparison. Starting from this point one may progress a little. The average count for dairy cows under all conditions is in the region of:—

Class of Animal.	R.C.	R.P.	R.P./R.C.	W.C.
Dairy Cows.....	6,660,000	32	4·7	9,400

Counts carried out early in pregnancy showed usually a higher red cell count than the average:—

Class of Animal.	Sex.	Period of Pregnancy.	R.C.	R.P.	W.C.
Bovine No. 79....	F.	1 month pregnant.....	6,900,000	29	10,400
Bovine No. 116...	F.	2½ months pregnant.....	6,880,000	33	7,000
Bovine No. 112...	F..	2½ months pregnant.....	7,200,000	34	7,200

But the variations met with later in pregnancy do not permit of any conclusions being drawn. The next table provides instances of these: —

Class of Animal.	Sex.	Period of Pregnancy.	R.C.	R.P.	W.C.
Bovine No. 116...	F..	5 months pregnant.....	6,660,000	33	9,000
Bovine No. 112...	F.	6 months pregnant.....	7,580,000	37	10,800
Bovine No. 79....	F.	4 months pregnant.....	6,420,000	32	11,200
Bovine No. 40 C..	F.	9 months pregnant.....	7,400,000	35	12,400

(*b*) Influence of Parturition.

Burnett states that parturition seems to lower the red cell count for a short time and, quoting himself and Traum, that in a bitch the red cell count remained low for two or more weeks after parturition. He also states that in the woman the count is lowered but should return to normal in from 10 to 14 days.

Most counts under this heading were done within one day of parturition: —

Class of Animal.	Time after Parturition.	R.C.	R.P.	R.P./R.C.
Female Bovine No. 126	1 day after parturition.....	6,960,000	38	5·5
Female Bovine No. 126	1½ months after parturition.	5,700,000	30	5·2
Female Bovine No. 126	2½ months after parturition.	5,600,000	30	5·3
Female Bovine No. 40c	1 hour after parturition.....	7,400,000	35	4·5
Female Bovine No. 40c	1 day after parturition.....	6,600,000	33	5·0
Female Bovine No. 158	1 day after parturition.....	8,020,000	36	4·5

The majority of cases showed a higher red cell count about the time of parturition than the average of 6,600,000 red cells per c.mm. This is in contrast to the claims of Burnett in connection with the influence of parturition of bitches and women. The percentage volume of red cells, immediately after calving, is also higher than the average of 32. The ratio of percentage volume of red cells to red cell count is also frequently higher than the average of 4.7.

(*c*) Conclusions.

1. It is practically impossible to obtain definite data on the influence of pregnancy unless an elaborate experiment is undertaken with a large number of animals.

2. Compared with the average red cell count of cows under all conditions it appears as if, at the onset of pregnancy, there is a rise in the number of red cells for a time; the count then appears to drop slightly until the time of actual parturition, when it again rises.

3. Red cell counts carried out within a day of calving showed a higher count than the average.

6. INFLUENCE OF OESTRUM.

One was unfortunate in that, at the time of this work, most of the cows at the Institution were pregnant but where oestrum appeared red cell counts were carried out. Only animals showing definite signs of "heat" were worked on. Only a few counts having been made it is difficult to draw any definite conclusions.

The red counts appear to be higher than the average on the day of oestrum in the majority of cows examined and on the day after slightly higher still, dropping again to nearer the average in about 10 days' time:—

Class of Animal.	Period.	R.C.	R.P.	R.P./R.C.
Bovine Female No. 73.	Oestrum.....	7,500,000	30	4·7
Bovine Female No. 73.	1 day after oestrum.....	5,840,000	29	4·9
Bovine Female No. 73.	14 days after oestrum.....	6,570,000	33	5·0
Bovine Female No. 11.	Oestrum.....	6,720,000	35	5·2
Bovine Female No. 11.	1 day after oestrum.....	6,850,000	32	4·6
Bovine Female No. 112	Oestrum.....	7,100,000	33	4·6
Bovine Female No. 112	1 day after oestrum.....	7,300,000	35	4·8
Bovine Female No. 112	11 days after oestrum.....	6,240,000	33	5·2
Bovine Female No. 82.	Oestrum.....	5,740,000	30	5·2
Bovine Female No. 82.	1 day after oestrum.....	6,840,000	35	5·1
Bovine Female No. 82.	10 days after oestrum.....	6,600,000	35	5·2
Bovine Female No. 1..	Oestrum.....	7,890,000	34	4·3

7. INFLUENCE OF ALTITUDE.

This was another phase of the subject that was touched on. Counts of a few animals were carried out at Potchefstroom, Transvaal, 4,436 feet above sea level and at Cedara, Natal, 3,541 feet above sea level—a difference in altitude of almost 1,000 feet. The cattle at both institutions were kept under almost identical conditions. Burnett (1908) states that "the majority"—of investigators—"have found that there is a considerable increase in the number of red corpuscles and of specific gravity in animals and men living at high altitudes." The difference in height above sea level between the two places is not great, but there do appear to be differences in red cell counts. However, it would not be possible to say these were due entirely to difference in altitude.

Class of Animal.	Age.	Institution.	R.C.
Bovine Female No. 20.	12 years.....	Potchefstroom.....	7,080,000
Bovine Female No. 9..	14 years.....	Cedara.....	5,500,000
Bovine Female No. 112	5 years.....	Potchefstroom.....	7,500,000
Bovine Female No. 136	5 years.....	Cedara.....	5,700,000
Bovine Female No. 142	11 months.....	Potchefstroom.....	9,920,000
Bovine Female No. 3..	11 months.....	Cedara.....	7,220,000

8. INFLUENCE OF LACTATION.

As pointed out previously, the normal condition of dairy cows should be defined as the pregnant one. To obtain results pertaining to the effects of lactation, these animals would need to be prevented from conceiving for at least a year. Owing to this difficulty, a series of counts was not done. R. J. M. Buchanan states that lactation tends to diminish the red cell counts. Da Costa (1905) states, however, that lactation is accompanied by a normal count in healthy, robust individuals. Both these references are to women.

An opportunity was taken to determine the red cell count of a few pregnant cows in milk and to correlate the milk yield per month with these counts. In addition there is the factor of natural "drying off" to be contended with. The "drying off" factor is seen on consulting the table below.

Class of Animal.	Month.	Milk Yield.	R.C.
Bovine Female No. 116.....	January.....	984¼ lb.	—
	February.....	874½ lb.	—
	Mareh.....	862¼ lb.	—
	April.....	795 lb.	6,880,000
	May.....	724 lb.	6,460,000
	June.....	561½ lb.	6,000,000
	July.....	472¾ lb.	6,600,000
Bovine Female No. 112.....	January.....	980 lb.	—
	February.....	832¼ lb.	—
	March.....	894¾ lb.	7,200,000
	April.....	797½ lb.	7,320,000
	May.....	807¾ lb.	7,620,000
	June.....	494½ lb.	6,720,000
	July.....	67 lb.	7,580,000

A few counts were carried out within an hour after milking and then just prior to the following milking, the interval of time between the two processes being about 9 hours.

Class of Animal.	Milking.	R.C.
Bovine Female No. 105.....	Before.....	5,740,000
	After.....	6,060,000
Bovine Female No. 117.....	Before.....	6,110,000
	After.....	6,250,000
Bovine Female No. 87.....	Before.....	6,180,000
	After.....	6,210,000

9. THE LEUCOCYTES OF CATTLE.

The generally recognized classification of the white cells of bovines has been followed, viz:—

(*a*) Lymphocytes.
(*b*) Monocytes.
(*c*) Neutrophiles.
(*d*) Eosinophiles.
(*e*) Basophiles.

Included with (*a*) is a variety of white cell termed by du Toit (1916) Reizzellen. In the same article this author discusses also the literature pertaining to the classification of white cells. As pointed out by Neser in his work on equine blood, a slight overstaining of the smear is an advantage. This applies equally well to the staining of bovine blood smears. Du Toit described each variety of white cell and one would simply quote him for reference purposes.

In unstained smears the leucocytes appear as opaque bodies usually larger than an average sized red blood cell and they contain nuclei which appear to have a greenish tint. One is able at times to distinguish between one or two varieties, e.g. neutrophiles and lymphocytes. For differential work, however, they must be stained.

(*a*) *Lymphocytes.*—In the majority of blood smears from normal cattle this is the variety that predominates in number. The nucleus is either round, oval or slightly irregular and occupies usually the greater part of the cell. Now and again one meets with lymphocytes that have apparently two or three round and oval nuclei, which give one the impression of being separate but in close opposition to each other. In some cases the nucleus is so large that one is able to make out only a small rim of the cytoplasm of the cell. The nucleus stains a dark purple and appears to be more homogeneous than the nuclei of monocytes when stained with May Grunewald and Giemsa. Occasionally isolated darker spots may be seen in the nucleus—these being probably nucleoli. The cytoplasm stains faintly blue and appears to be made up of a very fine substance. Round the periphery of the cell, when the nucleus is not large, one often sees a number of granules which vary in size and shape. If these granules are of a large size they are usually round and few in number, but if small they are often irregular in size and fairly numerous.

Reizzellen.—These are fairly large cells, as large as a large lymphocyte; the majority of them are oval but round ones are occasionally met with. The nucleus which is usually situated centrally in the cells stains similarly to that of a lymphocyte. The cytoplasm of the cell is coarse in structure and stains a dark blue colour. In young animals of both sexes there may be found in smears a number of these cells.

(*b*) *Monocytes.*—These cells, as a rule, are larger than the average lymphocytes seen in cattle blood. Their nucleus is, as a rule, irregular in outline but one meets with nuclei that vary from the normal, being oval or round in form. At times one is apt to have difficulty in distinguishing between a monocyte with a large round nucleus and but little cytoplasm and a typical lymphocyte. Thayer (1894) states that where one meets with typical monocytes and compares them with lymphocytes one must resort to the comparison of the nuclei rather than the size of the cell body. The structure of the nucleus of these cells is very characteristic—it can be described as appearing to be in sections somewhat like portions of a jig-saw puzzle. The cytoplasm stains a darker blue than that of the lymphocytes and appears to be coarser in texture. Vacuolation has been observed fairly frequently in the monocytes of adult bovines and occasionally small network bodies were seen in the cytoplasm. These small bodies stained a faint pink and are probably evidence of a degeneration. This is also mentioned by Burnett.

(*c*) *Neutrophiles.*—Cells of this variety are fairly constant in size and one has no difficulty in recognizing them on account of their characteristic nucleus. In well-stained smears by May Grunewald and Giemsa the nucleus takes a deep purple colour while the cytoplasm is neutrophilic. Distributed regularly through the cytoplasm are a number of granules which stain a faint red. The shape of the nucleus is very irregular.

(*d*) *Eosinophiles.*—These cells are about the size of a monocyte and again their appearance is so typical that one cannot mistake them in a stained smear. Their nucleus takes up a fair share of the cell and appears to have two lobes. The granules present are packed closely together and are not as large as those found in eosinophiles of the horse. Zietschmann, quoted by du Toit, states that the granules of eosinophiles of cattle measure on the average .5μ, while those of equines may reach a size of 2.9μ. In some preparations—especially observed in stained blood smears from veld cows— the granules take on a dirty dark red colour as against a light pink colour in the majority of stained blood smears of bovines.

(*e*) *Basophiles.*—These cells are about the same size as eosinophiles. The nucleus usually stains only faintly while the granules take on a dark colouration. In shape the nucleus varies from a curved one to one possessing several lobes. In the blood of bovines this variety of white cell is the rarest.

The Numbers.

One found the white cell count of bovines varied greatly among animals of about the same age and kept under similar conditions. Neser had the same experience in his work on the white cell count of equines. Du Toit also quotes numerous authorities to bear this out.

Numbers of White Cells.	Maximum.	Minimum.	Number of Animals Examined.	Authorities.
7,860 per c.mm.	—	—	—	Du Toit (1916).
8,241 per c.mm.	9,869 per c.mm.	6,217 per c.mm.	Average of 10 cows	Störch (1901).
8,580 per c.mm.	9,838 per c.mm.	8,047 per c.mm.	Average of 5 working cows	Störch (1901).
7,841 per c.mm.	9,992 per c.mm.	5,432 per c.mm.	Average of 10 bulls	Störch (1901).
9,367 per c.mm.	—	—	Average of 8 oxen	Störch (1901).
8,922 per c.mm.	11,046 per c.mm.	6,814 per c.mm.	Average of 19 cows	Schultz (1904).
7,031 per c.mm.	7,306 per c.mm.	6,458 per cmm..	Average of 7 oxen	Schultz (1904).
7,597 per c.mm.	10,312 per c.mm.	4,822 per c.mm.	—	Utendorfer(1907)
5,486 per c.mm.	10,610 per c.mm.	2,349 per c.mm.	—	Dimock & Thompson (1906).
9,000 per c.mm.	11,000 per c.mm.	7,000 per c.mm.	—	Refik-Bey (1902)
8,000 per c.mm.	—	—	—	Goodall (1909).
9,730 per c.mm.	—	—	—	Smith & Kilbourne (1893).

Several of these investigators also worked on the numbers of white cells in bovines of different ages and sex.

Class of Animals.	Sex.	Age.	W.C.	Authority.
Bovines...	Females.	12 days.....	15,130 per c.mm.	Du Toit (1916).
Bovines...	Male....	8 months.....	12,600 per c.mm.	Du Toit (1916).
Bovines...	Male....	10 months.....	12,100 per c.mm.	Du Toit (1916).
Bovines...	Unknown	3 hours.....	21,488 per c.mm.	Ströch (1901).
Bovines...	Unknown	8 to 14 days.....	12,800 per c.mm.	Ströch (1901).
Bovines...	Unknown	8 to 19 months..	10,100 to 14,900 per c.mm.	Ströch (1901).
Bovines...	Unknown	6 to 8 weeks.....	13,800 to 14,900 per c.mm.	Schultz (1904).
Bovines...	Unknown	Young cattle.....	11,000 to 13,000 per c.mm.	Schultz (1904).
Bovines...	Bulls....	Adult.....	7,841 per c.mm.	Störch, by Burnett (1908).
Bovines...	Oxen....	Adult.....	9,367 per c.mm.	Störch, by Burnett (1908).
Bovines...	Females.	Adult.....	8,241 per c.mm.	Störch, by Burnett (1908).
Bovines...	Unknown	Young adult.....	11,614 per c.mm.	Störch, by Burnett (1908).
Bovines...	Unknown	Calves.....	15,739 per c.mm.	Störch, by Burnett (1908).

The following white cell counts for age and sex were obtained in this work:—

Class of Animals.	Sex.	Age.	W.C.	Number of Animals Examined.
Bovines...	Females.	1 day to 13 months.....	13,000 per c.mm.	111
Bovines...	Females.	14 months to young adult.....	14,300 per c.mm.	14
Bovines...	Male....	1 day to 13 months.....	12,000 per c.mm.	73
Bovines...	Male....	14 months to young adult.....	9,900 per c.mm.	12
Bovines...	Male....	Oxen.....	9,000 per c.mm.	43
Bovines...	Female..	Young adults.....	10,700 per c.mm.	27
Bovines...	Female..	Adults (pregnant).....	9,400 per c.mm.	35
Bovines...	Female..	Adults (dry).....	9,500 per c.mm.	13
Bovines...	Female..	Adults (veld).....	11,000 per c.mm.	27

Taking the average white cell count for adult animals under all conditions as 9,700 per c.mm., we find that the young animals have a high white cell count which gradually decreases with advancing age. Comparing the average white cell counts of young male bovines from birth to young adult with that of young female bovines for the same period of age, one will notice that the females have a higher count than males:—

Class of Animals.	Sex.	Age.	W.C.
Bovine.....	Male....	Birth to 13 months.....	12,000 per c.mm.
Bovine.....	Female..	Birth to 13 months.....	13,000 per c.mm.
Bovine.....	Male....	14 months to young adult.....	9,900 per c.mm.
Bovine.....	Female..	14 months to young adult.....	14,300 per c.mm.

Of cattle under all conditions veld-reared cows show an average white cell count higher than any other class of adult stock, viz: 11,000 white cells per c.mm.

The variations encountered in bovines of different ages were marked. In the table below these figures may be seen:—

Class of Animal.	Sex.	Age.	Max. W.C. Count.	Min. W.C. Count.
Bovines...	Females.	Birth to 13 months.....	21,000 per c.mm.	5,500 per c.mm.
Bovines...	Females.	14 months to young adult	22,300 per c.mm.	9,600 per c.mm.
Bobines...	Males....	Birth to 13 months.....	19,600 per c.mm.	6,200 per c.mm.
Bovines...	Males....	14 months to young adult	13,300 per c.mm.	8,000 per c.mm.
Bovines...	Males....	Oxen.....	14,800 per c.mm.	6,300 per c.mm.
Bovines...	Females.	Adults.....	19,900 per c.mm.	5,000 per c.mm.

Differential Counts.

In this part of the work one again adopted Neser's methods in the actual counting. At least 200 cells were counted in each case and were recorded on a special sheet drawn up for the purpose. Just as one finds variations in the total white cell counts so does one find them in the differential counts of white cells. The numbers recorded are, of course, percentages.

Differential Counts.

Class of Animal.	Sex.	Age.	Lymphocytes.			Monocytes.			Neutrophiles.			Eosinophiles.			Basophiles.		
			Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr.
			%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Bovine...	M.	1 day to 13 months	83·0	24·5	68·0	8·5	1·0	4·0	68·0	12·5	30·0	16·0	0·0	2·0	3·0	0·0	1·0
Bovine...	M.	14 months to young adult	77·5	36·5	59·8	8·0	1·0	4·0	55·5	16·0	29·0	20·0	1·0	7·0	3·0	0·0	1·0
Bovine...	F.	1 day to 13 months	77·0	46·0	61·0	6·0	2·0	3·0	48·5	13·0	32·0	10·0	0·0	3·0	2·0	0·0	1·0
Bovine...	F.	14 months to young adult	71·0	41·0	54·0	6·0	1·0	4·0	47·0	21·0	33·0	16·0	4·0	8·0	2·0	0·0	1·0
Bovine...	M.	Oxen.....	70·0	36·0	51·0	13·0	2·0	6·0	56·0	16·0	30·0	23·0	3·0	12·0	2·0	0·0	1·0
Bovine...	F..	Adult (pregnant)...	72·0	34·0	53·0	9·0	2·0	4·0	57·0	16·0	33·0	23·0	3·0	9·0	2·0	0·0	1·0
Bovine...	F..	Adult (oestrum)....	65·0	27·0	—	8·0	3·0	—	47·0	22·0	—	22·0	4·0	—	1·0	0·0	1·0
Bovine...	F..	Adult (veld).....	62·0	29·0	48·0	9·0	3·0	4·0	44·0	18·0	30·0	33·0	6·0	17·0	2·0	0·0	1·0
Bovine...	F.	Adult(before milking)	66·0	28·0	—	8·0	2·0	—	55·0	16·0	—	20·0	6·0	—	2·0	0·0	—
Bovine...	F.	Adult (after milking)	62·0	33·0	—	7·0	2·0	—	50·0	18·0	—	23·0	5·0	—	1·0	1·0	—
Bovine...	F.	Adult (dry).....	71·0	27·0	53·0	7·0	3·0	4·0	59·0	21·0	34·0	15·0	1·0	8·0	2·0	0·0	1·0

The maximum, minimum and average differential white cell counts for equines are given by Neser as:—

Lymphocytes.			Monocytes.			Neutrophiles.			Eosinophiles.			Basophiles.		
Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr.	Max.	Min.	Avr
% 45·0	% 30·0	% 38·0	% 8·0	% 2·0	% 4·0	% 60·0	% 45·0	% 53·0	% 9·0	% 3·0	% 4·0	% 3·0	% 0·0	% 1·0

The Effect of Pregnancy and Parturition on the White Cell Count.

Störch found no increase in leucocytes in pregnant cows. It would appear that during the course of pregnancy there are fluctuations in the white cell counts at varying times, but the average count remains fairly constant.

Class of Animal.	Sex.	Period of Pregnancy.	W.C.
Bovine No. 116.....	F.	2½ months.....	7,000
Bovine No. 116.....	F.	3½ months.....	9,400
Bovine No. 116.....	F.	4 months.....	8,800
Bovine No. 116.....	F.	5 months.....	9,000
Bovine No. 82.....	F.	2 months.....	6,400
Bovine No. 82.....	F.	4 months.....	9,100
Bovine No. 82.....	F.	5½ months.....	8,500
Bovine No. 136.....	F.	8 months.....	10,100
Bovine No. 136.....	F.	9 months.....	9,200
Bovine No. 40c.....	F.	9 months (6 hours before calving)....	12,400

In the last case Bovine 40 C. there was a marked excess of Neutrophiles over Lymphocytes.

The Effect of Exercise on the White Cell Count.

Burnett states that a considerable increase in the number of leucocytes has been found after severe muscular exercise. Neser pointed out that the white cell counts of horses made from jugular blood always increased with fast work and in slow work it showed usually no change but occasionally an increase. The results obtained in this work showed that oxen that have plenty of exercise have an average white cell count lower than dairy cows which have very little real exercise. More contradictory still is the fact that veld cows show an average white cell count of 11,000 per c.mm., the highest of all adult cattle. Apparently other factors play a part in controlling the white cell count.

The Effect of Oestrum on the White Cell Count.

Buchanan states that there is a slight increase in the white cell counts of women at the time of menstruation and this is confirmed by other workers. This increase is said to be due to a greater number of eosinophiles being present.

In cattle oestrum appears to be craracterized by a change in the total white cell counts.

Class of Animal.	State.	W.C.
Bovine No. 147.....	Oestrum.....	13,600 per c.mm
Bovine No. 151.....	Oestrum.....	17,000 per c.mm.
Bovine No. 52.....	Oestrum.....	11,800 per c.mm.
Bovine No. 37.....	Oestrum.....	14,400 per c.mm.
Bovine No. 136.....	Oestrum.....	11,600 per c.mm.
Bovine No. 73.....	Oestrum.....	11,800 per c.mm.
Bovine No. 11.....	Oestrum.....	13,600 per c.mm.
Bovine No. 112.....	Oestrum.....	10,900 per c.mm.

This table shows a marked increase over the average white cell count, but on the other hand some cows may show a decreased count.

Class of Animal.	State.	W.C.
Bovine No. 49.....	Oestrum.....	9,700 per c.mm.
Bovine No. 67.....	Oestrum.....	8,400 per c.mm.
Bovine No. 117.....	Oestrum.....	9,100 per c.mm.
Bovine No. 46.....	Oestrum.....	9,400 per c.mm.

It would appear that the majority of cows in this state do show an increased white cell count.

Within from 10 to 14 days these counts show a decrease in many cases.

Class of Animal.	State.	W.C.
Bovine No. 49.....	14 days after oestrum.....	6,100 per c.mm.
Bovine No. 117.....	18 days after oestrum.....	6,400 per c.mm.
Bovine No. 73.....	14 days after oestrum.....	10,300 per c.mm.
Bovine No. 112.....	11 days after oestrum.....	7,600 per c.mm.

In addition to the increase in the total number of leucocytes, there are also changes in the differential counts of the white cells. The differential counts of white cells on the day of oestrum show an increased percentage of neutrophiles; in a number of cases there may be actually a greater percentage of neutrophiles than lymphocytes. The day after oestrum the percentage of neutrophiles once more drops

while counts done 10 to 14 days later often reveal a further drop in neutrophile percentage. The eosinophile count is increased in some cases.

Class of Animal.	State.	Lymphoeyte.	Neutrophile.	Eosinophile.
Bovine No. 67.....	Oestrum.....	47·5	38·5	8·0
Bovine No. 67.....	1 day after oestrum.	58·5	34·5	4·5
Bovine No. 67.....	14 days after oestrum	59·5	31·5	4·0
Bovine No. 52.....	Oestrum.....	33·5	44·5	20·5
Bovine No. 52.....	1 day after oestrum	50·5	26·5	20·0
Bovine No. 52.....	7 days after oestrum	52·0	22·5	23·0
Bovine No. 37.....	Oestrum.....	42·5	45·0	7·5
Bovine No. 37.....	1 day after oestrum	48·5	39·5	7·5
Bovine No. 37.....	20 days after oestrum	38·5	28·0	30·5
Bovine No. 117.....	Oestrum.....	38·5	43·0	11·5
Bovine No. 117.....	1 day after oestrum	48·5	25·5	22·0
Bovine No. 117.....	18 days after oestrum	57·0	20·0	20·0

The Effect of Lactation on the White Cell Count.

Da Costa states that lactation of itself causes no increase of white cells. In blood taken from cattle immediately after milking and then again prior to the following milking—the interval between the two milkings being about 9 hours—there is an increase of white cells. The eosinophiles also increase in many cases before milking than after milking.

Class of Animals.	State.	W.C.	Eosinophiles.
Bovine No. 105.....	After milking.....	12,400	14·5
Bovine No. 105.....	Before foll. milking.....	14,200	16·5
Bovine No. 89.....	After milking.....	12,600	8·5
Bovine No. 89.....	Before foll. milking.....	13,800	10·0
Bovine No. 52.....	After milking.....	10,200	10·5
Bovine No. 52.....	Before foll. milking.....	11,000	13·5

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APPENDIX.

In the tables and in the work the following symbols have been used:—

R.P. for the percentage volume of the red corpuscles.

R.C. for the number of red cells per c.mm. of blood.

M. for male.

F. for female.

W.C. for the number of leucocytes per c.mm. of blood.

Max. for maximum.

Min. for minimum.

Avr. for average.

Mths. for months.

TABLE 1.—YOUNG MALE ANIMALS FROM 1 DAY TO 13 MONTHS OLD.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
In Pen.										
Fries. Calf—										
Male V.....	4 hours..	—	10,620,000	—	—	—	—	—	—	—
In Pen.										
Fries. Calf—										
Male V.....	8 days..	—	10,580,000	—	—	—	—	—	—	—
In Pen.										
Fries. Calf—										
Male V.....	14 days.	—	10,205,000	—	—	—	—	—	—	—
In Pen.										
Fries. Bull Calf—										
D.O.A. 151.....	1 day...	51	11,480,000	4·4	11,500	31·0	5·0	63·5	0·5	—
In Pen.										
Fries. Bull Calf—										
D.O.A. 151.....	12 days.	45	11,100,000	4·1	10,700	50·0	8·0	40·5	0·5	0·5
In Veld.										
Fries. Bull Calf—										
D.O.A. 151.....	2 months	37	9,820,000	3·7	15,100	56·5	5·5	37·0	—	—
In Pen.										
D.O.A. 1.....	1 day...	34	7,860,000	4·3	8,000	49·5	7·0	43·0	—	0·5
In Pen.										
Fries. Grade—										
D.O.A. 1.....	3 days..	35	7,560,000	4·6	8,400	70·0	8·5	20·5	—	1·0
In Veld.										
Fries. Grade—										
D.O.A. 1.....	21 days.	36	8,360,000	4·3	17,700	28·0	3·5	68·0	—	0·5
In Pen.										
Fries. Bull Calf—										
D.O.A. 150.....	1 day...	23	6,440,000	3·6	8,700	24·5	5·0	68·0	2·5	—
In Pen.										
Fries. Bull Calf—										
D.O.A. 150.....	9 days..	24	6,480,000	3·7	8,700	48·0	5·0	44·5	0·5	1·5
In Veld.										
Fries. Bull Calf—										
D.O.A. 150.....	22 days.	31	8,120,000	3·8	11,600	67·5	7·5	25·0	—	—
Fries. Bull Calf—										
D.O.A. 149.....	15 days.	35	8,800,000	3·9	14,300	52·5	2·0	39·5	4·0	1·5
	30 days.	35	9,180,000	3·8	12,000	55·0	7·5	37·0	—	0·5
	7 weeks.	39	10,500,000	3·7	12,500	64·0	5·0	30·0	—	1·0
	8 weeks.	35	10,040,000	3·5	13,500	58·0	6·0	34·5	—	1·5
Africander Bull Calf—										
D.O.A. 107.....	3 months	32	8,400,000	3·7	15,900	—	—	—	—	—
	4 months	35	9,000,000	3·8	14,300	61·0	3·0	35·5	0·5	—
	5 months	32	8,680,000	3·7	11,200	65·0	4·5	30·0	—	0·5
	5¾ months	31	8,200,000	3·7	12,100	76·5	7·5	16·0	—	—
	6 months	29	8,400,000	3·5	9,000	80·5	4·5	15·0	—	—
S.A. Bull Calf—										
D.O.A. 64.....	3 months	40	10,420,000	3·8	7,100	77·5	1·5	12·5	6·5	2·0
	6 months	38	9,680,000	3·9	7,400	73·0	5·5	14·5	5·5	1·5
	7 months	32	8,300,000	3·8	13,500	80·5	4·0	13·0	1·5	1·0
	7¾ months	34	8,700,000	3·9	9,600	76·0	5·5	17·0	1·0	0·5
	8 months	33	8,200,000	4·0	8,700	75·0	1·5	18·5	5·0	—
Fries. Bull Calf—										
D.O.A. A.....	2 months	—	10,000,000	—	—	—	—	—	—	—
	2 months	—	10,008,000	—	—	—	—	—	—	—
	2 months	—	10,032,000	—	—	—	—	—	—	—

TABLE 1.—YOUNG MALE ANIMALS FROM 1 DAY TO 13 MONTHS OLD (*continued*).

Animal.	Age.	R.P.	R.C.	R.P. / R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Bull Calf—										
D.O.A. B.....	3 months	—	9,920,000	—	—	—	—	—	—	—
	3 months	—	9,620,000	—	—	—	—	—	—	—
Fries. Bull Calf—										
D.O.A. 148.....	4 months	33	9,780,000	3·4	9,300	73·0	4·0	22·0	1·0	—
D.O.A. 147.....	7½months	33	9,000,000	3·7	13,400	75·0	2·0	13·0	9·0	1·0
D.O.A. 146.....	7½months	38	10,000,000	3·8	15,800	54·0	2·0	39·0	5·0	—
D.O.A. 145.....	8 months	37	9,600,000	3·8	11,900	83·0	2·0	15·0	—	—
D.O.A. 144.....	8½months	33	8,200,000	4·0	14,100	64·0	1·0	28·0	6·0	1·0
	10 months	34	8,920,000	3·8	13,400	57·0	2·0	23·0	16·0	2·0
	12 months	37	8,220,000	4·5	16,800	—	—	—	—	—
D.O.A. 143.....	9 months	33	8,580,000	3·8	15,200	74·0	2·0	20·0	1·0	3·0
D.O.A. D.....	9 months	—	8,592,000	—	—	—	—	—	—	—
D.O.A. 142.....	10 months	35	8,000,000	4·3	10,500	76·0	2·0	20·0	2·0	—
D.O.A. 147.....	11 months	33	8,580,000	3·8	12,300	72·0	4·5	19·0	3·5	1·0
No Exercise for 3 *Mths.*										
Fries. Bull Calf—										
D.O.A. 139.....	11 months	—	7,912,000	—	—	—	—	—	—	—
Exercise for 11 *Mths.*										
Fries. Bull Calf—										
D.O.A. 130.....	11 months	—	9,120,000	—	—	—	—	—	—	—
No Exercise for 3 *Mths.*										
Fries. Bull Calf—										
D.O.A. 134.....	12 months	—	7,192 000	—	—	—	—	—	—	—
Exercise for 12 *Mths.*										
Fries. Bull Calf—										
D.O.A. 135.....	12 months	—	10,090,000	—	—	—	—	—	—	—
D.O.A. 135.....	13 months	—	9,120,000	—	—	—	—	—	—	—
D.O.A. 142.....	13 months	36	8,800,000	4·0	12,400	62·5	0·5	32·5	3·0	1·5
Ayrshire Bull—										
D.O.A. "Clifton"	12 months	35	8,680,000	4·0	16,400	68·5	3·0	18·5	10·0	—
Fries. Bull Calf 4...	6 months	37	10,500,000	3·7	15,100	68·0	4·0	21·0	6·0	1·0
	6½months	34	9,200,000	3·7	13,300	63·5	4·5	25·5	6·0	0·5
	8 months	33	9,000,000	3·6	19,600	49·5	7·0	38·0	4·0	1·5
	9 months	36	9,700,000	3·7	11,800	72·0	6·0	22·5	3·0	1·5
	10 months	33	8,690,000	3·8	13,900	70·0	3·0	21·0	6·0	—
Fires. Bull Calf 9...	5 months	35	9,400,000	3·6	12,400	72·0	5·5	21·5	0·5	0·5
	6 months	35	9,400,000	3·7	11,000	60·5	4·5	30·0	5·0	—
	7 months	33	8,800,000	3·7	16,200	65·0	6·5	25·0	2·5	1·0
	8 months	30	7,300,000	4·1	15,200	73·5	5·0	18·5	2·5	0·5
Fries. Bull.....	8 days..	40	9,600,000	4·1	9,400	57·0	6·5	35·5	—	1·0
Fries. Bull Calf 2...	6½months	30	8,000,000	3·7	9,200	54·0	4·0	34·5	6·5	1·0
	7 months	30	7,800,000	3·8	8,700	52·5	2·5	39·5	4·5	1·0
	7¾months	32	8,400,000	3·8	17,300	51·5	6·0	34·5	6·5	1·5
	8½months	31	7,500,000	4·1	8,600	66·0	6·0	25·0	2·0	1·0
	9¾months	30	8,000,000	3·7	7,600	66·0	4·5	26·0	3·0	0·5
Cross Bred Bull Calf	2 months	35	9,400,000	3·7	7,800	82·0	0·0	18·0	0·0	0·0
	1 month.	25	9,600,000	3·6	7,200	54·5	4·5	40·0	1·0	—
	1½month.	40	10,300,000	3·8	8,500	64·5	0·5	32·0	2·0	1·0
	1¾month.	35	9,400,000	3·7	6,700	62·5	9·0	27·5	—	1·0
	3 weeks.	40	10,400,000	3·8	7,500	50·0	18·0	32·0	—	—
Fries. Bull 3.....	6 months	28	7,200,000	3·8	13,400	51·0	5·0	41·0	2·0	1·0
	7 months.	30	7,800,000	3·8	8,700	52·5	2·5	39·5	4·5	1·0
	8 months	30	7,200,000	4·1	16,100	53·5	5·0	35·5	5·0	1·0
	8¾months	32	7,200,000	4·4	12,900	64·5	7·0	24·0	3·5	1·0
	10 months	25	5,580,000	4·4	10,600	71·0	4·0	23·5	1·5	—
	10 months	29	5,900,000	4·9	10,300	—	—	—	—	—

TABLE 1.—YOUNG MALE ANIMALS FROM 1 DAY TO 18 MONTHS OLD (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Bull 11.....	3 weeks.	38	9,400,000	4·0	11,000	47·5	11·5	38·5	1·0	1·5
	1 month.	37	10,000,000	3·7	8,500	37·5	6·0	54·0	2·0	0·5
	2 months	39	10,800,000	3·6	10,200	77·0	7·5	12·0	1·0	2·5
	3 months	37	10,500,000	3·5	12,400	67·0	4·5	24·5	3·5	0·5
	$4\frac{1}{2}$months	34	9,500,000	3·5	15,500	63·5	7·5	25·5	1·5	2·0
Cross Bred Bull.....	3 weeks.	40	10,300,000	3·8	8,800	62·5	6·0	31·5	—	—
Fries. Bull 12.....	7 days..	35	8,860,000	3·9	9,400	50·5	4·5	43·5	1·0	0·5
	$3\frac{1}{2}$ weeks	37	10,000,000	3·7	6,200	71·5	8·0	19·5	—	1·0
	2 months	39	10,000,000	3·9	7,100	69·0	5·0	23·5	2·0	0·5
	$2\frac{3}{4}$months	38	11,400,000	3·6	9,600	69·0	7·0	22·0	2·0	—
	$3\frac{3}{4}$months	36	11,100,000	3·2	11,300	64·5	7·5	25·0	3·0	—

TABLE 2.—YOUNG MALE ANIMALS FROM 14 MONTHS TO 36 MONTHS.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Bull—										
D.O.A. 143.....	14 mths.	35	8,640,000	4·0	11,900	77·5	1·0	16·0	2·5	3·0
D.O.A. 136.....	14 mths.	—	7,856,000	—	—	—	—	—	—	—
D.O.A. 145.....	15 mths.	37	9,000,000	4·0	13,300	72·0	1·0	25·0	1·5	0·5
Sussex Bull—										
D.O.A. 137.....	18 mths.	34	6,980,000	4·9	9,200	76·0	2·0	21·0	0·5	0·5
	19 mths.	40	8,220,000	4·9	10,000	56·5	3·5	35·0	3·0	2·0
D.O.A. 136.....	18 mths.	37	7,900,000	4·6	9,500	68·5	4·0	23·5	2·5	1·5
	19 mths.	37	8,000,000	4·6	9,400	64·5	3·0	26·5	5·5	0·5
Orfords.....	19 mths.	40	9,600,000	4·1	8,000	62·0	4·5	30·0	2·5	1·0
Grade Bull—										
2.....	30 mths.	33	6,910,000	4·7	11,500	47·0	4·5	32·0	16·0	—
3.....	32 mths.	33	6,790,000	4·7	9,700	46·5	5·5	27·0	20·0	0·5
4.....	35 mths.	31	6,940,000	4·5	8,100	69·5	8·0	16·0	5·0	0·5
16.....	31 mths.	34	7,210,000	4·7	8,300	52·5	7·5	33·5	9·0	0·5
21.....	33 mths.	33	6,960,000	4·7	11,200	36·5	5·0	55·5	3·0	—

Table 3.—Young Female Animals from Birth up to and including 13 Months.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Heifer—										
D.O.A. 5.....	1 day...	44	10,820,000	4·0	15,100	50·0	2·5	47·0	—	0·5
D.O.A. 158.....	4 days..	46	10,700,000	4·3	10,400	46·5	2·5	46·5	3·5	1·0
D.O.A. 161.....	5 days..	40	9,060,000	4·4	12,500	46·0	3·5	48·5	2·0	—
D.O.A. 161.....	1 mth...	39	10,330,000	3·9	12,400	54·5	3·0	42·0	0·5	—
Kept in Pen.										
Fries. Heifer—										
D.O.A. VI.....	1 day...	—	10,960,000	—	—	—	—	—	—	—
	3 days..	—	10,030,000	—	—	—	—	—	—	—
	7 days..	—	9,680,000	—	—	—	—	—	—	—
	12 days.	—	9,265,000	—	—	—	—	—	—	—
D.O.A. VII.....	1 day...	—	9,964,000	—	—	—	—	—	—	—
	3 days..	—	9,504,000	—	—	—	—	—	—	—
	7 days..	—	9,336,000	—	—	—	—	—	—	—
	10 days.	—	9,448,000	—	—	—	—	—	—	—
	15 days.	—	9,176,000	—	—	—	—	—	—	—
D.O.A. 159.....	2 weeks.	34	8,930,000	3·8	13,200	57·5	4·5	37·0	0·5	0·5
D.O.A. 159.....	3 weeks.	44	10,880,000	4·0	12,800	49·0	3·5	44·0	2·5	1·0
D.O.A. 160.....	5 weeks.	39	12,060,000	3·2	10,500	53·0	2·0	45·0	—	—
D.O.A. 149.....	2 mths..	36	9,500,000	3·7	16,100	61·5	4·5	31·0	1·0	2·0
D.O.A. 149.....	3 mths..	33	9,720,000	3·4	8,200	71·0	2·0	25·0	—	2·0
D.O.A. 4x.....	2 mths..	42	11,100,000	3·7	21,000	58·5	4·0	35·5	2·0	—
Grade I, Cedara...	2 mths..	46	11,840,000	3·8	13,500	70·0	3·0	26·0	1·0	—
D.O.A. 91, Cedara	4 mths..	47	13,000,000	3·6	13,800	77·0	4·5	13·0	5·0	0·5
D.O.A. 157.....	4 mths..	40	10,690,000	3·7	13,900	50·5	5·5	33·5	10·0	0·5
D.O.A. 89c.....	5 mths..	43	12,260,000	3·5	18,600	53·5	6·0	37·5	2·5	0·5
D.O.A. 153.....	5 mths..	30	7,390,000	4·0	12,000	63·5	3·5	30·5	1·0	1·5
D.O.A. 153.....	5 m. 2 wks	27	7,730,000	3·5	10,600	75·0	5·0	20·0	—	—
D.O.A. 147.....	6 mths..	40	10,900,000	3·7	14,000	61·5	4·5	32·0	1·0	1·0
D.O.A. 151.....	7 mths..	34	9,160,000	3·7	12,300	55·5	5·5	36·5	1·5	1·0
D.O.A. 90c.....	8 mths..	33	8,410,000	3·9	12,200	66·5	2·0	29·0	2·0	0·5
D.O.A. 144.....	8 mths..	35	9,400,000	3·7	19,000	—	—	—	—	—
	8 m. 1 wk.	38	10,240,000	3·8	11,900	77·0	3·0	17·0	3·0	—
	10 mths.	32	8,900,000	3·6	12,300	70·0	2·0	20·0	8·0	—
D.O.A. 149.....	10 m. 3 wks	32	7,760,000	4·1	11,300	56·0	2·0	30·5	10·0	1·5
D.O.A. G. 3.....	11 mths.	31	7,220,000	4·3	16,200	75·0	3·0	19·5	6·1	1·0
D.O.A. 142.....	11 mths.	41	9,920,000	4·1	15,600	59·0	5·0	33·0	3·0	—
	12 mths.	37	8,900,000	4·1	10,200	72·0	3·0	17·0	8·0	—
	13 mths.	29	7,520,000	3·8	10,900	66·5	1·5	24·5	6·5	1·0
Fries. Heifer—										
11.....	±1 mth..	47	11,870,000	3·9	12,400	—	—	—	—	—
	3½ mths.	38	11,000,000	3·6	12,100	65·5	5·5	27·5	1·0	0·5
	4¼ mths.	35	9,500,000	3·6	10,800	55·5	5·5	37·5	0·5	1·0
	6 mths..	35	9,200,000	3·8	11,600	52·0	5·0	36·5	4·5	2·0
	6½ mths.	36	9,700,000	3·7	11,400	61·5	3·5	34·0	—	1·0
	7 mths..	33	9,600,000	3·3	11,600	58·0	5·0	36·0	0·5	0·5
20.....	5½ weeks	44	11 600 000	3·8	11,000	54·5	4·5	40·0	1·0	—
5.....	±1 mth..	40	10,360,000	3·8	17,000	48·5	3·5	47·5	—	0·5
2.....	±1 mth..	35	9,170,000	3·8	5,500	67·5	6·5	24·0	0·5	1·5
	2 mths..	37	10,000,000	3·7	5,700	58·0	3·0	36·0	1·0	2·0
	3½ mths.	35	10,000,000	3·5	6,900	67·0	8·0	24·0	—	1·0
	5½ mths.	35	9,500,000	3·7	6,300	59·5	3·5	35·0	—	2·0
	6 mths..	34	9,400,000	3·6	12,400	54·5	6·5	33·5	3·0	2·5
	6½ mths.	33	10,000,000	3·3	10,100	57·5	2·5	34·0	5·5	0·5
	7 mths..	35	9,500,000	3·7	7,900	64·0	5·0	28·0	1·0	2·0

TABLE 3.—YOUNG FEMALE ANIMALS FROM BIRTH UP TO AND INCLUDING 13 MONTHS (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries Heifer—										
1	1 mth...	39	11,000,000	3·5	6,500	63·5	4·0	26·5	5·5	0·5
	2 mths..	43	11,000,000	3·9	7,100	76·5	3·5	17·5	2·0	0·5
	3½ mths.	33	9,000,000	3·6	7,000	60·0	9·5	29·5	0·5	0·5
	5½ mths.	29	8,800,000	3·3	9,500	61·0	2·5	34·0	1·5	1·0
	6 mths..	29	7,900,000	3·6	13,000	45·5	5·5	48·5	—	0·5
	6½ mths.	34	9,200,000	3·7	8,700	64·0	5·0	26·0	3·5	1·5
	7 mths..	34	8,800,000	3·9	12,500	58·5	3·5	35·5	1·5	1·0
3	1 mth...	39	10,700,000	3·7	8,700	70·5	6·0	20·5	3·0	—
	2 mths..	35	8,120,000	4·3	9,900	43·0	3·5	51·0	2·0	0·5
4	1 mth...	50	13,300,000	3·7	13,100	54·0	3·0	43·0	—	—
	3½ mths.	36	9,600,000	3·7	13,200	78·0	5·0	17·0	—	—
7	1 mth...	39	10,300,000	3·8	8,000	72·0	4·5	22·0	1·0	0·5
	2 mths..	33	8,600,000	3·8	10,400	60·0	3·5	34·5	1·0	1·0
	4¾ mths.	30	7,500,000	4·0	10,100	57·0	4·5	37·0	—	1·5
	6 mths..	31	8,200,000	3·8	8,400	63·0	5·5	31·0	—	0·5
	6½ mths.	35	8,500,000	4·1	9,400	58·0	5·5	34·5	1·5	0·5
	7 mths..	33	8,400,000	3·9	11,000	69·5	3·5	24·5	—	2·5
9	1 mth...	47	12,310,000	3·8	10,900	46·5	1·5	51·5	—	0·5
	2 mths..	37	9,900,000	3·8	15,000	40·0	6·0	54·0	—	—
	3½ mths.	35	9,600,000	3·6	7,400	59·5	6·0	33·5	—	1.0
	5½ mths.	33	7,900,000	4·0	11,300	46·0	8·5	45·0	—	0·5
	6 mths..	30	8,200,000	3·6	11,000	56·0	3·5	36·5	2·0	2·0
	7 mths..	34	9,100,000	3·7	10,900	54·5	5·5	38·0	0·5	1·5
8	2 mths..	31	7,660,000	4·0	11,800	65·5	5·5	28·5	0·5	—
	3½ mths.	30	8,300,000	3·7	12,500	54·5	2·0	41·5	1·5	0·5
	5½ mths.	33	9,200,000	3·6	13,500	69·5	3·0	26·0	0·5	1·0
	6 mths..	34	9,400,000	3·6	10,000	55·5	3·0	38·5	2·0	1·0
	6½ mths.	33	8,800,000	3·7	10,400	59·5	3·0	36·0	1·0	0·5
	7 mths..	29	7,200,000	4·0	13,600	64·0	6·0	30·0	—	—
13	2 mths..	30	7,820,000	3·8	8,900	49·0	3·0	38·0	9·0	1·0
	5 mths..	29	8,800,000	3·3	9,700	54·0	3·0	39·0	2·0	2·0
	6 mths..	31	8,600,000	3·6	15,100	35·0	3·5	54·5	6·5	0·5
12	5½ mths.	33	9,200,000	3·6	11,300	67·5	3·5	26·0	0·5	2·5
	6 mths..	32	8,700,000	3·7	11,000	68·0	4·5	24·0	1·5	2·0
	6½ mths..	30	8,700,000	3·5	11,300	68·5	7·5	19·5	3·0	1·5
	7 mths..	25	5,600,000	4·4	6,300	79·0	7·0	13·0	0·5	0·5
	7½ mths.	28	6,200,000	4·5	12,400	59·5	6·5	34·0	—	—
	7¾ mths.	30	6,700,000	4·4	10,500	—	—	—	—	—
17	5½ mths.	36	10,000,000	3·6	17,100	50·0	2·5	29·5	18·0	—
	7 mths..	35	9,400,000	3·7	10,300	61·5	5·5	28·5	4·0	0·5
	7½ mths.	34	8,600,000	3·9	7,500	68·5	6·5	23·0	2·0	—
	8½ mths.	33	9,200,000	3·5	15,600	64·0	4·5	25·0	5·5	1·0
23	7½ mths.	29	7,300,000	3·9	8,600	61·5	6·0	26·5	5·5	0·5
	8½ mths.	31	7,100,000	4·3	8,400	52·0	3·0	42·5	1·5	1·0
	8¾ mths.	33	8,400,000	3·9	11,200	50·0	4·5	39·0	6·0	0·5
	9 mths..	32	7,200,000	4·4	6,200	82·5	9·0	6·0	1·5	1·0
16	6½ mths.	35	10,200,000	3·5	11,600	64·5	5·0	27·5	2·5	0·5
	8 mths..	36	9,000,000	4·0	14,400	75·0	5·5	0·18	1·5	—
	8½ mths.	34	10,000,000	3·4	11,200	70·5	4·0	20·5	5·0	—
	9½ mths.	32	8,900,000	3·6	10,900	67·5	4·0	25·5	2·0	1·0
15	4 mths..	32	7,100,000	4·5	13,500	53·5	3·5	40·5	0·5	2·0
	5 mths..	33	8,400,000	3·9	14,400	64·0	6·5	26·0	2·5	1.0
	6 mths..	33	7,800,000	4·2	13,500	69·5	4·5	22·5	2·0	1·5

TABLE 3.—YOUNG FEMALE ANIMALS FROM BIRTH UP TO AND INCLUDING 13 MONTHS (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries Heifer—										
14.....	4 mths..	35	7,900,000	4·4	14,100	60·5	5·0	28·5	5·0	1·0
	5 mths..	31	7,900,000	3·9	13,400	52·0	4·5	36·0	7·5	—
	6 mths..	35	7,800,000	4·4	14,900	71·0	4·5	19·5	4·0	1·0
20.....	2 mths..	33	8,700,000	3·8	12,000	52·0	9·5	33·0	4·5	1·0
10.....	2 mths..	37	8,600,000	4·3	10,800	56·0	5·0	36·5	2·0	0·5
18.....	2 weeks.	51	12,200,000	4·1	14,400	37·0	4·5	57·0	—	1·5
	3 weeks..	51	11,900,000	4·3	12,100	49·0	2·0	47·5	1·0	0·5
	4 mths..	50	12,000,000	4·1	13,400	58·0	5·0	36·0	0·5	0·5
	5 mths..	43	11,400,000	3·7	11,800	82·5	9·0	6·0	1·5	1·0
	6 mths..	40	11,200,000	3·5	12,400	—	—	—	—	—
30.....	6½ mths.	31	9,400,000	3·3	8,700	55·5	7·5	34·5	2·0	0·5
	7 mths..	32	8,800,000	3·6	9,800	46·0	2·0	47·0	5·0	—

TABLE 4.—YOUNG FEMALE ANIMALS FROM 14 MONTHS TO YOUNG ADULTS.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Ayrshire Heifer—										
D.O.A. 63.....	14 mths.	31	6,600,000	4·6	9,600	59·5	3·5	30·0	7·0	—
Fries. Heifer—										
D.O.A. 17.....	14 mths.	35	7,810,000	4·4	17,600	62·5	3·1	21·5	13·0	—
D.O.A. 143.....	14 mths.	31	7,800,000	3·9	13,900	57·0	1·0	32·5	8·0	1·5
	17 mths.	38	8,690,000	4·3	16,700	51·5	4·0	35·5	8·0	1·0
	33 mths.	38	7,400,000	5·1	14,200	43·5	3·5	28·5	25·5	—
Fries. Heifer G.—										
D.O.A. 115.....	15 mths.	33	7,480,000	4·4	17,900	70·5	1·5	21·5	6·5	—
D.O.A. 16.....	15 mths.	29	6,120,000	4·7	19,900	41·0	1·0	47·0	10·0	1·0
Fries. Heifer—										
D.O.A. 137.....	15 mths.	36	7,900,000	4·4	12,400	65·0	3·0	24·0	6·0	1·0
	18 mths.	35	8,500,000	4·1	12,700	50·5	2·5	42·0	4·0	1·0
Calved 1 day—An Accidental Service.										
Fries. Heifer—										
D.O.A. 144.....	16 mths.	31	7 150 000	4·3	22,300	62·0	3·0	24·0	10·5	0·5
	17 mths.	30	7,230,000	4·1	14,700	48·5	4·0	29·5	16·0	2·0
	17 mths. 3 weeks	32	7,300,000	4·3	13,700	64·5	5·5	29·5	5·0	0·5
	33 mths.	40	7,600,000	5·2	9,400	61·5	6·0	20·0	12·0	0·5
D.O.A. G. 12.....	18 mths.	34	7,340,000	4·6	15,000	60·5	1·0	26·0	12·0	0·5
D.O.A. 142.....	21 mths.	34	7,500,000	4·5	12,300	56·0	2·5	33·5	8·0	—
Ayrshire Heifer—										
D.O.A. 60.....	23 mths.	30	7,120,000	4·2	15,700	70·5	4·0	20·5	5·0	—

Table 5.—Dry Cows.

Animal.	Age.	R.P.	R.C.	R.P. / R.C.	W.C.	L.	M.	N.	E.	B.
	Calved.									
Fries. Cow—										
D.O.A. 126.....	1 day...	38	6,960,000	5·5	11,300	63·0	2·5	32·5	1·5	0·5
D.O.A. 126.....	1½ mths.	30	3,700,000	5·2	9,400	42·0	4·5	38·5	14·5	0·5
D.O.A. 49c.....	1 hour..	35	7,400,000	4·5	12,400	34·0	6·0	57·0	3·0	—
D.O.A. 40c.....	1 day...	33	6,600,000	5·0	7,100	63·0	5·5	20·5	10·5	0·5
D.O.A. G. 143c...	6 days..	32	6,600,000	4·8	5,000	71·0	5·0	21·0	1·0	2·0
D.O.A. 116.....	1 mth...	30	5,700,000	5·2	9,400	42·0	4·5	38·5	14·5	0·5
D.O.A. 126	2½ mths.	30	5,600,000	5·3	10,000	55·0	3·0	37·5	4·0	0·5
D.O.A. 46.....	2 mths..	29	5 370,000	5·4	9,400	26·5	4·0	59·0	9·5	1·0
Oestrum.....	—	32	62·4	5·1	—	—	—	—	—	—
Fries. Cow—										
D.O.A. 128.....	1 mth...	32	6,160,000	5·1	12,400	46·5	6·5	39·5	7·0	0·5
D.O.A. 5.....	11/11/26 Adult... 21/12/26	33	6,240,000	5·0	7,700	59·5	2·5	24·5	12·0	1·5
D.O.A. 112.....	Adult...	35	7,100,000	4·9	10,200	59·0	6·0	28·0	7·0	—
	14/3/27	37	7,000,000	5·2	11,400	69·0	2·5	22·0	5·0	1·5
D.O.A. 84.....	11/11/26	32	6,530,000	4·9	9,600	49·0	2·5	38·5	9·0	1·0

Table 6.—Adult Cows (Dairy)—Pregnancy.

Animal.	Age.	R.P.	R.C.	R.P. / R.C.	W.C.	L.	M.	N.	E.	B.
	Pregnant for									
Fries. Cow—										
D.O.A. 116.....	2½ mths.	33	6,880,000	4·7	7,000	57·0	6·5	27·5	8·0	1·0
	3½ mths.	30	6,460,000	4·6	9,400	50·0	1·5	38·5	9·5	0·5
	4 mths..	28	6,000,000	4·6	8,800	54·0	2·0	36·0	7·5	0·5
	5 mths..	33	6,660,000	4·9	9,000	58·0	2·5	31·5	7·5	0·5
D.O.A. 112.....	2½ mths.	34	7,200,000	4·7	8,200	62·0	3·0	31·0	4·0	—
	3 mths..	37	7,320,000	5·0	7,100	72·0	4·0	19·0	3·0	2·0
	4 mths..	37	7,600,000	4·8	11,400	64·0	1·5	22·0	14·0	—
	5 mths..	33	6,720,000	4·9	10,800	67·0	3·5	20·5	7·5	1·5
	6 mths..	37	7,580,000	4·9	9,500	65·5	5·0	21·5	7·0	1·0
D.O.A. 82.....	2 mths..	26	6,400,000	4·1	6,400	42·0	3·5	40·5	13·5	0·5
	4 mths..	30	6,180,000	4·9	9,100	44·5	2·0	44·0	9·5	—
	5½ mths.	32	6,460,000	4·9	8,500	61·0	5·0	25·5	7·5	1·0
D.O.A. 79.....	1 mth...	29	6,900,000	4·2	10,400	44·5	1·5	43·0	9·5	1·0
	4 mths..	32	6,420,000	4·9	11,200	56·5	4·0	32·0	6·5	1·0
Fries. Grade, Cedara—										
D.O.A. 136.....	8 mths..	24	5,700,000	4·2	10,100	63·0	3·5	23·0	10·5	—
	9 mths..	26	5,510,000	4·7	9,200	58·0	6·5	28·0	7·0	0·5
Fries. Cedara—										
D.O.A. 7.....	8 mths..	25	5,270,000	4·8	7,400	51·5	9·0	29·5	14·5	0·5
	17 y. 9 m.									
Fries. Cow—										
D.O.A. 40c.....	9 mths..	35	7,400,000	4·7	12,400	34·0	6·0	57·0	3·0	—
This cow calved 6 hrs	after being	bled.								

TABLE 6.—ADULT COWS (DAIRY).—PREGNANCY (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Cow—										
D.O.A. 67.....	2 mths..	33	7,260,000	4·6	9,000	63·0	3·0	23·0	11·0	—
D.O.A. 129.....	2 mths..	37	7,310,000	5·0	8,900	45·5	2·5	36·5	15·0	0·5
D.O.A. 37.....	4 mths..	35	6,930,000	5·0	13,500	51·5	5·5	27·0	16·0	—
	4 m. ½ d.	33	6,800,000	4·8	11,900	49·5	7·5	23·0	20·0	—
D.O.A. 49.....	3 mths..	34	6,960,000	4·9	9,900	62·0	4·0	24·0	9·5	0·5
	3 m. ½ d.	34	6,460,000	5·2	11,800	52·0	4·5	37·5	6·0	—
Ayrshire Cow—										
D.O.A. 117.....	13 days.	30	6,250,000	4·8	9,400	54·0	6·5	34·5	4·5	0·5
	13½ days	31	6,110,000	5·0	9,500	54·0	4·0	34·0	7·5	0·5
Fries. Cow—										
D.O.A. 89.....	3 mths..	30	6,720,000	4·4	12,600	40·5	2·5	48·5	8·5	—
	3 m. ½ d.	33	6,810,000	4·8	13,800	49·0	3·0	38·0	10·0	—
D.O.A. 119.....	5 mths..	26	5,300,000	4·9	6,100	53·5	4·5	33·5	8·5	—
	5 m. ½ d.	—	—	—	—	4·7	5·5	36·0	11·0	0·5
D.O.A. 52.....	4 days..	30	5,640,000	5·3	10,200	50·0	6·5	32·5	10·5	0·5
	4½ days.	30	5,600,000	5·3	11,000	42·0	4·5	40·0	13·5	—
Fries. Grade—										
D.O.A. 171.....	2 mths..	34	6,780,000	5·0	10,300	66·0	4·0	15·5	14·5	—
	2 m. ½ d.	34	6,700,000	5·0	10,100	61·0	2·0	22·5	14·0	0·5
D.O.A. 87.....	2½ mths.	31	6,180,000	5·0	16,100	61·0	4·0	17·5	17·0	—
	2½ m. ½ d.	30	6,200,000	4·9	14,400	56·0	3·5	17·5	22·5	0·5
Fries. Cow—										
109.....	Adult...	33	6,790,000	4·9	8,000	43·0	3·0	33·5	19·5	1·0
49.....	Adult...	33	6,620,000	4·9	9,200	50·0	4·5	40·0	5·5	—
37.....	Adult...	33	6,940,000	4·7	9,000	45·0	3·0	32·5	18·5	1·0
	21/12/26									
49.....	Adult...	33	6,760,000	4·9	7,700	64·0	0·5	21·5	13·5	0·5
	21/12/26									
	Pregnant for									
67.....	1 mth...	32	6,600,000	4·8	8,300	29·5	2·0	52·5	16·0	—
	21/12/26									
52.....	1 mth...	31	6,180,000	5·0	10,900	44·5	6·0	34·0	15·5	—
	19/1/27									
52.....	2 mths..	34	6,500,000	5·2	10,700	43·0	10·0	21·5	24·5	1·0
	19/1/27									
67.....	2 mths..	29	5,600,000	5·1	9,600	35·5	3·5	41·0	20·0	—
	19/1/27									
49.....	2 mths..	35	6,860,000	5·0	11,300	51·5	3·5	29·0	15·0	1·0
	2/3/27									
67.....	3 mths..	36	7,000,000	5·1	9,900	59·5	3·0	28·0	9·0	0·5
	2/3/27									
52.....	2½ mths.	34	6,700,000	5·0	13,000	54·0	2·5	30·0	13·5	—
	2/3/27									
37.....	2½ mths.	36	7,400,000	4·9	11,300	49·5	4·5	40·5	5·0	0·5
	2/3/27									
109.....	2½ mths.	37	7,300,000	5·0	8,400	43·0	6·0	44·0	6·5	0·5
	9/3/27									
48.....	2½ mths.	35	7,000,000	5·0	13,400	52·5	3·5	25·0	7·5	1·5
	15/3/27									
Ayrshire Cow—										
117.....	2½ mths.	34	6,400,000	5·3	8,800	40·5	4·0	44·0	11·5	—
	15/3/27									

TABLE 6.—ADULT COWS (DAIRY).—PREGNANCY (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Cow—										
37	2½ mths. 15/3/27	35	7,400,000	4·7	11,600	50·0	3·0	31·0	15·5	0·5
75	2½ mths. 15/3/27	33	6,400,000	5·1	8,200	50·5	4·5	31·0	12·0	2·0
140	2½ mths. 14/3/27	37	7,500,000	4·9	7,500	73·5	7·5	15·0	4·0	—
126	2½ mths. 20/3/27	32	6,200,000	5·1	6,000	62·0	4·5	26·0	6·0	1·5
49	2½ mths. 20/3/27	38	7,400,000	5·1	9,900	59·5	4·0	29·0	7·0	0·5
48	2½ mths. 20/3/27	34	6,700,000	5·0	11,300	62·5	55·0	23·5	7·0	1·5
67	4 mths.. 20/3/27	33	6,400,000	5·1	9,400	53·5	3·5	32·5	10·5	—
37	4 mths.. 20/4/27	32	7,400,000	4·3	11,000	48·0	3·0	38·5	10·5	—
37	4 mths.. 20/4/27	38	7,500,000	5·0	14,700	41·5	3·5	45·5	9·0	0·5
49	4 mths.. 20/4/27	43	8,000,000	5·3	10,400	61·5	5·0	31·5	2·0	—
52	5 mths.. 20/4/27	32	6,480,000	4·9	13,800	43·0	6·5	36·5	14·0	—
48	5 mths.. 20/4/27	34	7,400,000	4·6	10,000	60·0	3·5	20·5	15·5	0·5

TABLE 7.—OESTRUM.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Cow—										
D.O.A. 73	Oestrum...	30	7,500,000	4·7	11,800	50·0	3·5	37·0	9·5	—
	1 day after.	29	5,840,000	4·9	9,500	65·0	5·0	28·5	6·0	0·5
	14 days after	33	6,570,000	5·0	10,300	58·5	3·5	30·0	8·0	—
Fries. G. Cow—										
D.O.A. 11	Oestrum...	35	6,720,000	5·2	13,600	46·5	6·0	33·0	14·0	0·5
	1 day after	32	6,850,000	4·6	13,800	66·0	5·0	21·5	7·5	—
Fries. Cow—										
D.O.A. 112	Oestrum...	33	7,100,000	4·6	10,900	44·5	3·5	46·5	5·5	—
	1 day after.	35	7,300,000	4·8	10,700	53·5	3·0	39·5	4·0	—
	11 days after	33	6,240,000	52·0	7,600	56·0	8·0	26·0	9·0	1·0
D.O.A. 82	Oestrum...	30	5,740,000	5·2	9,800	41·0	4·5	45·0	8·5	0·5
	1 day after.	35	6,840,000	5·1	9,000	48·5	3·0	42·0	6·0	0·5
	10 days after	35	6,660,000	5·2	7,100	51·0	8·0	37·0	4·0	—
Veld Cow—										
1	Oestrum...	34	7,890,000	4·3	11,000	29·0	5·5	43·5	22·0	—
Fries. Cow—										
D.O.A. 46	Oestrum...	29	5,370,000	5·3	9,400	26·5	4·0	59·0	9·5	1·0

TABLE 7.—OESTRUM (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Cow—										
49.....	Oestrum...	35	6,910,000	5·0	9,700	53·0	2·0	40·0	3·5	1·5
	1 day after.	37	6,800,000	5·4	7,900	64·0	6·0	27·0	2·5	0·5
	14 days after	33	6,800,000	4·8	6,100	62·5	2·0	30·5	4·5	0·5
67.....	Oestrum...	33	6,680,000	4·9	8,400	47·5	5·0	38·5	8·0	1·0
	1 day after.	38	7,480,000	5·0	11,800	58·5	2·5	34·5	4·5	—
	14 days after	33	5,840,000	5·6	8,700	59·5	4·5	31·5	4·0	0·5
Fries. Heifer—										
151.....	Oestrum...	35	7,520,000	4·6	19,100	38·5	5·0	37·0	19·5	—
	1 day after.	37	8,740,000	4·2	19,900	46·5	2·0	31·5	20·0	—
	14 days after	—	—	—	—	—	—	—	—	—
Ayrshire Cow—										
117.....	Oestrum...	30	6,140,000	4·9	9,100	38·5	5·5	43·0	11·5	1·5
	1 day after.	36	6,500,000	5·5	7,800	48·5	3·5	25·5	22·0	0·3
	18 days after	34	6,860,000	4·9	6,400	57·0	1·0	20·0	20·0	2·0
Fries. Heifer—										
147.....	Oestrum...	33	6,930,000	4·7	13,600	48·5	3·5	35·0	13·0	—
	1 day after.	40	8,350,000	4·8	10,400	58·5	7·0	22·5	11·5	0·5
	13 days after	39	8,500,000	4·5	16,000	63·5	2·0	28·5	5·5	0·5
151.....	Oestrum...	30	7,120,000	4·3	17,100	43·5	14·0	41·0	11·0	0·5
	1 day after.	30	6,990,000	4·1	16,800	52·5	2·0	35·5	9·5	0·5
	13 days after	—	—	—	—	—	—	—	—	—
Fries. Cow—										
52.....	Oestrum...	32	6,020,000	5·3	11,800	33·5	1·5	44·5	20·5	—
	1 day after.	31	5,200,000	5·9	9,900	50·0	2·5	26·5	20·0	0·5
	7 days after	32	5,640,000	5·6	11,400	52·0	2·0	22·5	23·0	0·5
37.....	Oestrum...	33	6,400,000	5·1	14,400	42·5	4·5	45·0	7·5	0·5
	1 day after.	35	7,100,000	4·9	11,400	48·5	3·5	39·5	7·5	1·0
	20 days after	33	6,900,000	4·8	11,000	38·5	2·0	28·0	30·5	1·0
126.....	Oestrum...	35	6,500,000	5·3	11,600	48·5	4·5	43·5	3·0	0·5
	1 day after.	32	5,800,000	5·4	10,900	55·0	6·0	32·0	6·0	1·0
	15 days after	34	6,600,000	5·0	10,000	56·0	8·5	23·0	12·0	0·5

TABLE 8.—VELD COWS.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Veld Cow—										
1.....	Adult...	34	7,890,000	4·3	11,000	29·0	5·5	43·5	22·0	—
2.....	Adult...	30	7,070,000	4·2	12,800	40·0	3·5	40·5	16·0	—
3.....	Adult...	32	6,000,000	5·3	11,200	61·5	4·5	20·5	13·9	0·5
4.....	Adult...	24	4,890,000	4·9	7,600	42·5	4·5	39·0	14·0	—
5.....	Adult...	29	5,550,000	5·2	8,300	35·0	3·5	34·0	27·5	—
6.....	Adult...	32	6,930,000	4·6	12,300	58·5	9·0	27·0	5·5	—
7.....	Adult...	32	7,100,000	4·5	10,700	56·0	6·0	17·5	19·5	1·0
A8.....	Adult...	29	5,900,000	4·9	11,400	34·5	4·5	28·0	32·5	0·5
B9.....	Adult...	30	5,800,000	5·1	13,900	60·0	4·0	23·0	12·5	0·5
C10.....	Adult...	25	5,110,000	4·9	13,400	—	—	—	—	—
D11.....	Adult...	23	6,000,000	3·8	18,500	44·5	3·0	44·0	8·5	—

TABLE 8.—VELD COWS (*continued*).

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
E12	Adult...	30	6,090,000	4·9	9,200	42·5	4·0	23·5	28·0	2·0
F13	Adult...	25	5,860,000	4·3	9,400	48·5	2·5	25·5	27·5	1·0
G14	Adult...	28	6,430,000	4·4	8,300	53·0	3·5	28·5	15·0	—
H15	Adult...	24	5,220,000	4·6	9,100	54·5	5·5	30·5	9·0	0·5
I16	Adult...	23	5,410,000	4·2	8,000	51·5	5·0	21·0	22·0	0·5
J17	Adult...	28	6,270,000	4·5	11,200	55·0	2·5	32·5	9·5	0·5
X18	Adult...	25	5,050,000	4·9	12,600	39·0	5·0	42·0	13·0	1·0
Y19	Adult...	30	5,680,000	5·2	8,100	48·0	5·0	37·0	9·0	1·0
Bull—										
ZY20	Adult...	23	5,630,000	4·0	7,100	75·5	5·0	13·5	6·0	—
2x	Y.A....	30	6,780,000	4·4	17,300	43·5	4·0	40·5	11·5	0·5
Ox—										
IX	A.....	35	7,020,000	4·9	10,300	60·5	8·0	20·5	10·5	0·5
Veld Cow—										
F1	A.....	30	6,100,000	4·9	10,200	—	—	—	—	—
S1	A.....	35	7,300,000	4·7	11,000	—	—	—	—	—
S2	A.....	33	6,500,000	5·0	10,500	—	—	—	—	—
S3	A.....	33	6,800,000	4·8	8,300	—	—	—	—	—
S4	A.....	29	5,400,000	5·3	6,300	—	—	—	—	—

TABLE 9.—DAIRY COWS BEFORE (B) AND AFTER (A) MILKING.

Animal.	Age.	R.P.	R.C.	R.P./R.C.	W.C.	L.	M.	N.	E.	B.
Fries. Cow—										
D.O.A. 116 B	Adult...	—	—	—	—	53·5	4·5	28·5	12·0	1·5
A	Adult...	31	6,620,000	4·7	8,600	46·5	3·5	39·0	9·5	—
D.O.A. 119 B	Adult...	—	—	—	—	47·0	5·5	36·0	11·0	0·5
A	Adult...	26	5,300,000	4·9	6,100	53·5	4·5	33·5	8·5	—
D.O.A. 105 B	Adult...	28	5,740,000	4·9	14,200	27·5	1·5	54·5	16·5	—
A	Adult...	30	6,060,000	4·9	12,400	33·0	2·5	50·0	14·5	—
D.O.A. 89 B	Adult...	33	6,810,000	4·8	13,800	49·0	3·0	38·0	10·0	—
A	Adult...	30	6,720,000	4·4	12,600	40·5	2·5	48·5	8·5	—
Ayrshire Cow—										
D.O.A. 117 B	Adult...	31	6,110,000	5·0	9,500	54·0	4·0	34·0	7·5	0·5
A	Adult...	30	6,250,000	4·8	9,400	54·0	6·5	34·5	4·5	0·5
Fries. Cow—										
D.O.A. 49 B	Adult...	34	6,460,000	5·2	11,800	52·0	4·5	37·5	6·0	—
A	Adult...	34	6,960,000	4·9	9,900	62·0	4·0	24·0	9·5	0·5
D.O.A. 37 B	Adult...	33	6,800,000	4·8	11,900	49·5	7·5	23·0	20·0	—
A	Adult...	35	6,930,000	5·0	13,500	51·5	5·5	27·0	16·0	—
D.O.A. 52 B	Adult...	30	5,600,000	5·3	11,000	42·0	4·5	40·0	13·5	—
A	Adult...	30	5,640,000	5·3	10,200	50·0	6·5	32·5	10·5	0·5
Fries Grade—										
D.O.A. 171 B	Adult...	34	6,780,000	5·0	10,300	66·0	4·0	15·5	14·5	—
A	Adult...	34	6,700,000	5·0	10,100	61·0	2·0	22·5	14·0	0·5
D.O.A. 87 B	Adult...	31	6,180,000	5·0	16,100	61·0	4·0	17·5	17·0	0·5
A	Adult...	30	6,210,000	4·9	914,400	56·0	3·5	17·5	22·5	0·5

Table 10.—Working Oxen.

Animal.	Age	R.P.	R.C.	R.P. / R.C.	W.C.	L.	M.	N.	E.	B.
Grade Ox. C—										
61	Adult...	32	8,320,000	5·0	10,800	57·5	4·0	21·5	16·0	1·0
64	Adult...	32	7,140,000	4·5	8,600	47·0	6·0	37·5	9·0	0·5
49	Adult...	35	8,180,000	4·1	6,400	62·0	4·5	27·0	5·0	1·5
67	Adult...	33	7,500,000	4·4	7,300	43·0	5·0	37·5	14·5	—
2	Y. Adult	33	6,910,000	4·7	11,500	47·0	4·5	32·0	16·0	0·5
3x	Y. Adult	33	6,790,000	4·7	9,700	46·5	5·5	27·0	20·5	0·5
4	Y. Adult	31	6,940,000	4·5	8,100	69·5	8·0	16·0	5·5	1·0
16	Y. Adult	34	7,210,000	4·7	8,300	52·5	7·5	33·5	9·5	2·0
7	Adult...	40	8,180,000	4·8	10,100	47·5	8·0	36·5	8·0	—
9	Adult...	38	8,280,000	4·6	9,500	47·5	9·0	24·5	19·0	—
28	Adult...	38	7,830,000	4·8	10,700	52·5	7·5	25·5	14·5	—
19	Adult...	40	8,210,000	4·8	10,000	60·5	8·5	22·5	8·5	—
15	Adult...	38	7,760,000	4·8	11,400	47·5	7·0	29·5	15·0	1·0
22	Adult...	38	7,720,000	4·9	13,300	56·5	9·0	23·0	11·0	0·5
17	Adult...	43	9,300,000	4·6	11,400	51·5	7·0	27·0	14·5	—
21	Y.....	33	6,960,000	4·7	11,200	36·5	0·5	55·5	3·0	—
26	Adult...	41	8,770,000	4·7	14,800	52·0	9·0	26·0	12·0	1·0
62	Adult...	34	7,200,000	4·6	8,900	51·0	5·5	33·5	9·5	0·5
76	Adult...	26	5,480,000	4·8	7,600	43·5	8·0	36·5	11·5	0·5
70	Adult.	33	6,740,000	4·8	8,700	56·5	4·5	30·5	8·5	—
58	Adult...	32	7,000,000	4·5	7,600	40·0	6·0	49·5	4·0	0·5
65	Adult...	34	6,980,000	4·9	8,200	52·5	7·5	28·5	10·5	1·0
68	Adult...	31	6,860,000	4·6	8,300	44·5	12·5	29·5	12·5	1·0
66	Adutl...	29	6,000,000	4·8	7,400	49·5	9·0	32·5	8·5	0·5
61	Adult...	40	8,050,000	4·9	8,700	50·0	8·5	31·0	10·5	—
81	Adult...	34	7,630,000	4·4	9,200	45·0	6·5	16·0	31·0	1·0
81	Adult...	41	8,480,000	4·8	7,400	52·0	7·0	20·0	20·5	0·5
98	Adult...	34	7,520,000	4·5	7,400	56·0	5·0	33·5	4·5	1·0
55	Adult...	35	7,370,000	4·7	6,500	55·0	2·5	37·0	5·5	—
47	Adult...	32	6,550,000	4·9	6,300	49·5	7·0	25·5	16·5	1·5
84	Y.A....	30	6,290,000	4·7	7,500	51·0	10·5	26·5	11·0	1·0
97	A.....	30	6,440,000	4·6	7,800	57·0	5·5	28·5	8·5	0·5
Grade Ox—										
71	Adult...	35	7,250,000	4·8	9,400	55·5	5·5	26·5	14·5	—
48	Adult...	45	9,300,000	4·7	6,000	55·0	5·0	24·0	15·5	0·5
77	Adult...	34	7,200,000	4·7	7,300	46·0	6·0	25·5	22·5	—
60	Adult...	32	6,440,000	4·9	8,200	54·5	2·0	37·5	5·5	0·5
48	Adult...	36	8,300,000	4·3	6,700	48·5	5·5	33·5	12·0	0·5
A.A. 48	Adult...	36	7,480,000	4·8	7,200	47·0	4·0	35·5	13·0	0·5
49	Adult...	41	8,340,000	4·9	7,500	47·0	3·5	41·0	8·0	0·5
78	Adult...	36	8,400,000	4·4	11,200	56·0	3·5	32·5	7·5	0·5
16	Adult...	41	8,460,000	4·8	6,600	63·0	7·0	24·0	5·5	0·5
41	Adult...	34	7,140,000	4·7	7,900	51·5	5·5	28·5	14·0	0·5
57	Adult...	35	7,130,000	4·9	8,800	43·0	7·5	31·5	17·0	1·0

The Water Consumption of Sheep in a Nutrition Experiment at Onderstepoort.

By F. BRAUNS, B.Sc., B.Sc. Agric., Research Officer, Onderstepoort.

For an experiment with sheep on the effect of different quantities of phosphorus on wool growth, fertility, etc., as stated in an article by du Toit and others in this Report, it became necessary to determine the water consumption for controlling the intake of minerals of the animals. The sheep in the nutrition experiment, which is still continuing, are kept in a shed with open yards on either side and their feed consists of hay and maize endosperm, both of which are dry. Restricted movement and dry food under climatic conditions such as ours are a set of conditions peculiar to this experiment; this justifies the publication of the results obtained, as they will be required, no doubt, for future work here.

For reference Table 1 is given, which represents data on the subject obtained at Michigan and Colorado Agricultural Experimental Stations under the conditions of open yard feeding.

Table 1.

	Average Amount of Water Consumed Daily.	Weight of Water Consumed Daily.	Number of Trials.
Michigan Station.	Litres.	lb.	
Grain and clover-hay.....	0·63	1·44	8
Grain roots and clover-hay.....	0·85	1·9	3
Clover-hay and sugar-beet.....	0·13	0·3	1
Colorado Station.			
Grain and Alfalfa-hay.....	2·3 (cold water)	5·1	2
Grain and Alfalfa-hay.....	2·29 (warm water)	5·3	2

From Table 1 the effect of the nature of the food consumed on the daily consumption of water is apparent. The authors further reported that the sheep in confinement drank less water than those not subjected to restricted movement.

In the test carried out at this Institute, all the sheep in the nutrition experiment, 41 in number, were used. These happened to be divided into two groups as given in the tables, for each yard

respectively, on either side of the feeding shed so that the experiment was done in duplicate by force of circumstances, but fortunately all the same. The ration consisted of 550 grams maize endosperm and 300 grams of hay and the weights of the sheep were approximately 70 lb. Free access to the water troughs was allowed from 9.0 a.m. to 3.0 p.m. and the sheep were at liberty to remain in direct sunlight or in the shade. The yard, being only 12 by 120 feet, obviously restricted the movement of the sheep. The test was made in summer and carried out for 6 days on each occasion. The results are given in Tables 2 and 3.

TABLE 2.

	Amount of Water Consumed.	Average per Sheep per Day.	Mean Temperature during Day.	Sunshine, Hours per Day.
	Litres.	Litres.	°F.	Hours.
Group A: 25 Sheep....	34	1·3	67·7	11
	32	1·29	67·1	7·3
	38	1·52	69·8	9
	24	0·96	71·5	8·5
	28	1·12	76·1	9·5
	21	1·23	73·1	10·5
Averages.....	29·5	1·23	70·9	7·65
Group B: 16 Sheep....	17	1·06	67·6	11
	17	1·06	67·1	7·3
	20	1·25	69·8	9
	15	0·93	71·5	8·5
	22	1·37	76·1	9·5
	21	1·23	73·1	10·5
Averages.....	18·6	1·15	70·9	7·65

TABLE 3.

	Amount of Water Consumed.	Average per Sheep per Day.	Mean Temperature during Day.	Sunshine Hours per Day.
	Litres.	Litres.	°F.	Hours.
Group A: 25 Sheep....	38	1·65	74·5	7·3
	38	1·65	70·4	10·5
	38	1·65	73·5	10·5
	19	0·8	71·8	10·5
	38	1·65	69	4·5
	38	1·65	73·9	10·45
Average.....	36·5	1·5	78·8	8·96
Group B: 18 Sheep....	24	1·33	74·5	7·3
	26	1·4	70·4	10·5
	28	1·55	73·5	10·5
	24	1·3	71·8	10·5
	20	1·1	69	4·5
	24	1·3	73·9	10·45
Averages...	24·3	1·33	78·8	8·96

The average daily consumption of water for the different groups and for the two periods agrees fairly well. This could have been anticipated, for the climatic conditions were fairly constant. The daily water consumption for the first period (Table 2) is slightly lower than that for the second period (Table 3) but evidently the latter was the hotter period as the mean temperature and the total hours of sunshine indicate. The rations remained the same throughout the test.

Summary.

The daily water consumption of sheep kept under a condition of restricted movement on a ration of flaked maize and hay has been studied and found to be approximately 1.3 litres.

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Anatomical Studies, No. 14*: Anomalies of a Cross-bred Ram.

By Professor H. H. CURSON, F.R.C.V.S., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort and N. T. v. d. LINDE.

The following anomalies were observed in a young ram, No. 22,981, (Woolled Persian and Black Persian cross) born 12/11/28, and procured in April, 1929, through the kindness of the junior author. (Path. No. 9,345).

(*a*) Unilateral cryptorchidism involving the left testicle. In a study by Quinlan and Curson (1927), it was indicated that cryptorchisdism was a rare condition in the sheep. In the case in question the right testicle had been removed by castration at 5 weeks of age in the routine of a sheep farm, and although the left testis

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* Cases of anatomical interest encountered in the Anatomical Department of the Faculty of Veterinary Science (Transvaal University College) are published under this title. The following are previous contributions to the series:—

No. 1.—QUINLAN, J. B., and CURSON, H. H. "A rare case of cryptorchidism in a monorchid ram" (1927). *Vet. Jnl.*, Vol. 83, p. 579.

No. 2.—CURSON, H. H., JACKSON, C., and NEITZ, W. O. "A case of reduction of the thoracic vertebrae in a donkey" (1927). *S. Afr. Jnl. Sc.*, Vol. 24, pp. 450-1.

No. 3.—CURSON, H. H., and QUINLAN, J. B. "'Gut-tie' or strangulatio ducto-spermatica (Walch) in a merino wether" (1928). *Jnl. S. Afr. Med. Assn.*, Vol. 1, No. 2, p. 97.

No. 4.—CURSON, H. H. "An anomaly of the renal pelvis in a horse" (1928). *Vet. Rec.*, Vol. 8, p. 1006.

No. 5.—CURSON, H. H., and ROSSITER, L. W. "Ossification of the fascia lata in a horse" (1928). *Vet. Rec.*, Vol. 8, p. 1006.

No. 6.—CLAPHAM, W. S. B. "An unusual feature of the musculature in the shoulder of a donkey" (1929). *Vet. Rec.*, Vol. 9, p. 66.

No. 7.—CURSON, H. H. "Hypoplasia of a testicle and hyperplasia of the prostate in a dog" (1929). *15th Rpt. Dir. Vet. Serv.*, Vol. 2, p. 1185.

No. 8.—BARNARD, W. G. "An abnormal first lumbar vertebra in a horse" (1929). *Vet. Rec.*, Vol. 9, p. 675.

No. 9.—SCHOEMAN, J. H., and CLOETE, J. H. L. "Anomalies in a horse" (1929). *Vet. Rec.*, Vol. 9, p. 675.

No. 10.—BARNARD, W. G. "Ossification in the left diaphragmatic crus of a horse" (1929), *Vet. Jnl.*, Vol. 85, p. 355.

No. 11.—CURSON, H. H. "Anomaly of the thymus gland in a calf" (1929). *Jnl. S. Afr. Vet. Med. Assn.*, Vol. 1, No. 3, p. 59.

No. 12.—CURSON, H. H. "An anomaly of the left cornea in an ox" (1929). *Jnl. S. Afr. Vet. Med. Assn.*, Vol. 1, No. 3, p. 61.

No. 13.—CURSON, H. H. Persistent right ovidnct in a pullet" (1930). *Vet. Rec.*, Vol. 10, p. 138.

could not be felt, the fact that the animal posessed a fifth "limb" naturally attracted greater attention. In the dissecting room (12/6/29) the following picture was presented: Attached by a ligament to the left sterno-costal portion of the fleshy rim of the diaphragm (caudal aspect) was the retained testicle, and easily recognisable in the fold was the epididymis. Extending backwards from this in a spiral fashion was the rudiment of a vas deferens, approximately 8 cm. in length. The gland measured $4 \times 2.8 \times 1.5$ cm. and unlike other testicles of this type, was firm (See Fig. 1).

On microscopic examination (see Fig. 2) the typical histology of an arrest in spermatogenesis was observed, viz: absence of spermatids and spermatozoa in the seminal tubules and the presence of an abundance of interstitial cells. In many cases there appeared to be a separation of the seminal epithelium from the membrana propria, suggesting cast formation, while in other cases, the central cells of these "casts" had become disintegrated.

(*b*) The right kidney, instead of being bean-shaped in outline, was flattened dorsally, the lateral border was straight, and the ventral surface was markedly concave.

(*c*) The left lung was abnormally small, this condition being closely associated with the deformity described under (*e*).

(*d*) A fifth "limb," as will be observed in Figs. 3 and 4, was suspended from the skin covering the xiphoid region of the sternum and was rudimentary. The outline of the bony skeleton is shown by dotted lines in Fig. 1. There were indications at four places of attempts at joint formation. The proximal end of the bony structure was surrounded by a pad of fat. At birth this structure was only an inch from the ground, (standing position), but 7 months later it was $3\frac{1}{2}$ inches.

(*e*) The axial skeleton was curved in the thoracic region, the convexity being directed to the right and most prominent at the 5th, 6th and 7th vertebrae. As a result of this distortion (see Fig. 5) the dorsal spines pointed laterally and to the left.

Embryologically the diaphragmatic position of the testis may be explained as the result of failure of caudal migration. Instead of proceeding backwards and downwards to the scrotum, the gland in some peculiar way became anchored to the diaphragm by the cranial portion of the primitive mesorchium. With regard to (*d*), it is possible that a group of cells had become detached from the unsegmented somatic mesenchyme and later developed into a rudimentary limb. According to Arey (1925) the appendicular skeleton is apparently not derived from the sclerotomes.

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FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

Anatomical Studies, No. 15 : Accessory Spleens in a Horse.

By Professor H. H. CURSON, F.R.C.V.S., Dr. Med. Vet.,
Veterinary Research Officer, Onderstepoort.

THE object of this note is to record the presence of two small independent masses of splenic tissue situated in the dorsal portion of the Lig. gastro-lienale. These are marked A. and B. in Fig. I. C is a similar mass of tissue, but constriction from the main spleen in this case is not complete.

These accessory spleens were observed on 26/9/29 when horse (Gelding) 19,678 was destroyed on account of Horsesickness, and was examined post mortem. (See P.M. Report 8,574).

Anatomical Studies, No. 15.] [Curson.

FIG I.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

Anatomical Studies, No. 16 : On Two Cases of Free-Martinism.

By Professor H. H. CURSON, F.R.C.V.S., Dr. Med. Vet.,
Veterinary Research Officer, Onderstepoort.

Introduction.

Figures relating to twinning in cattle in South Africa are difficult to obtain especially details regarding birth of (*a*) normal females. (*b*) normal males, (*c*) normal male and normal female, and (*d*) normal male and free-martin. Crew (1925) shows " that twins are almost invariably binovular " in the bovine. and when bisexual and " vascular inter-communication becomes established, as it does in seven cases out of eight " (as a result of fusion of the chorions), a free-martin will be developed. Quinlan (1929) met with the condition in 6 females out of " over 500 examined for sterility," and in 3 of the cases a history of twinning was established. He quotes Keller's experience viz. only 6 per cent. females from a bisexual twin pregnancy with normal genitalia. Fincher and William's (1926) article on arrest in sexual development in the case of single births (heifers) should also be considered in a study of this nature.

Friesland " Heifer " 3,590 (Path. Spec. 9,418).

The above animal, aged 2, arrived from Potchefstroom School of Agriculture on 21/6/29 and was killed on 8/7/29. The Principal was unable to state whether the animal had ever shown oestrum (his E. 2/15 of 14/10/29).

As will be noted (see Fig. 1), the " heifer " possessed a typical female appearance, although the teats were unusually small.

On post mortem examination (see Fig. 2) the *ovaries* were found to be small and elongated (1.5 cm. × 0.5 cm.) and associated with the right ovary was a cyst with a diameter of 0.5 cm. The *Fallopian tubes* as such did not exist. On the right side a thin flexuous cord located in the postero-lateral aspect of the corresponding horn and measuring 4.5 cm. (the convolutions being disregarded) was taken to represent the right oviduct. The opening corresponding to the infundibulum tubae uterinae was present on both sides, being about 2.5 cm. in diameter. *Uterus.* The body and horns were present, the former measuring 2 cm. (length) × 1 cm. (breadth), and the latter 4 cm. × 2 cm. each. There was a definite lumen in which a glairy mucous material was present. Reaching backwards to the anterior extremity of the vagina was a fibrous structure 12 cm. in length. This was double at its posterior end resembling two crura and at the point where these united, a pair of dense fibrous cords

(A and B) each 8 cm. in length, arose and proceeded forwards on either side of the fibrous structure mentioned above. The *vagina* i.e. from the external urethral orifice to the anterior extremity of the vagina (origin of crura referred to above) measured 5.5 cm. in length and the diameter at the external urethral orifice was 4 cm. The vagina was cone-shaped, the apex pointing forwards. A strong fibrous band (F.B.) 0.5 cm. situated dorso-ventrally and in the middle line, guarded the vaginal opening, being situated 1 cm. anterior to the external urethral orifice. On either side of the base of the fold was a small opening (the left was double the size of the right) and a probe inserted into the left orifice proceeded forwards and laterally, between the mucosa and the vaginal muscle, towards two bodies (C and D) now to be described. The anterior body, club-shaped and 4.5 cm. in length, arose 1 cm. from the anterior extremity of the vagina, near the base of the crus mentioned previously. The posterior body, 1.5 cm. in length, arose 2 cm. behind the anterior structure. On the opposite side similar symmetrical bodies (E and F) were to be seen. The channels along which it was possible to pass the probe are the canals of Gärtner, (C.G.) the remnants of the embryonic Wolffian ducts. On the lateral side of each of the entrance to Gärtner's canals was a small cul-de-sac proceeding forwards under the mucosa for a distance of 2 cm. The *vulva* was 12 cm. in length and the diameter approximated that of the initial part of the vagina. Besides the suburethral diverticulum, there were two further medially placed cul-de-sacs, the first 6 cm. behind the external urethral orifice and the second 1.5 cm. behind this again. The former was 1 cm. in depth whereas the latter was 3 cm. deep and situated immediately in front of an unusually well developed clitoris. On either side of the middle line of the floor 2 pairs of pouches were present, the posterior apparently leading from the glandulae vestibulares majores (G.V.M.) Very little mammary tissue was present.

FRIESLAND "HEIFER" 2,971 (Path. Spec. 9,419).

"Heifer" 2,971, born 29/10/27, arrived from Glen School of Agriculture on 10/5/29. Her appearance was normally female as is indicated by her photograph. (See Fig. 3.) According to the Principal, the animal was never observed to show oestrum (his E. 28 of 30/7/29). On post-mortem examination (8/7/29), the *ovaries* were seen to be abnormal, the right, 2.5×2.5 cm. in diameter, being cystic. It also presented what appeared to be an old corpus luteum. The left ovary was small and elongated being 1 cm. $\times$ 1.75 cm. In both cases the counterpart of the infundibulum tubae uterinae was present, the slit-like opening (I.T.U.) being 0.5 cm. in diameter. Nothing representing the *oviducts* was seen, thus accounting for the gap, (see Fig. 4), between the right uterine horn and the right ovary. The connection on the left side was made up of peritoneum and connective tissue. In the mesosalpinx on either side was a lymph gland-like body, 0.75 cm. in diameter. This on histological examination showed a firm mass of fibrous connective tissue containing several cavities lined by cubical epithelium (See Fig. 5). This structure, the paroöphoron, is a remnant of the caudal portion of the Wolffian duct and is homologous with the paradidymis

in the male. The *uterus* was represented by a diminutive body and horns, the body being 11 cm. long × 1 cm. broad, and the horns 5 × 1.5 cm. Throughout the uterus, as described above, a lumen, filled with clear mucous material, was present. A probe is shown inserted in the body. Extending backwards from the uterine body to the apex of the vagina was a fibrous cord varying in diameter from 0.3-0.7 cm. At its left anterior aspect was a thick dense fibrous structure 2 cm. in length (A) and at the right posterior aspect was a similar, but larger (3 cm.) structure (B). The *vagina* was cone-shaped, being 5 cm. in length by 4.5 cm. at its entrance. At the apical extremity was a pair of firm club-shaped fibrous bodies (C and D) which projected outwards and slightly forwards. The left was 6.5 cm. × 1.5 cm. (at the middle) and the right 1 cm. longer. The inner surface of the apex showed two pits, each 1 cm. deep and these appeared formerly to have communicated with the structures C and D. Immediately to the front and right of the external urethral orifice was a channel which undoubtedly represented the right canal of Gärtner (C.G.). The *vulva* was 9 cm. in length and the diameter was approximately 4.5 cm. Besides the suburethral diverticulum there was a well marked fossa 1 cm. in depth in front of the clitoris, which was apparently normal. On either side of the middle ventral line a pouch apparently leading from the glandulae vestibulares majores (G.V.M.) was present.

The mammary tissue was well developed and the teats were normal in size.

Each of the specimens described above constitutes the more usual type of free-martinism. It must be remembered, however, that cases showing extreme modification in the male direction also occur (Fraser Roberts and Greenwood 1928).

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FIG. 1.

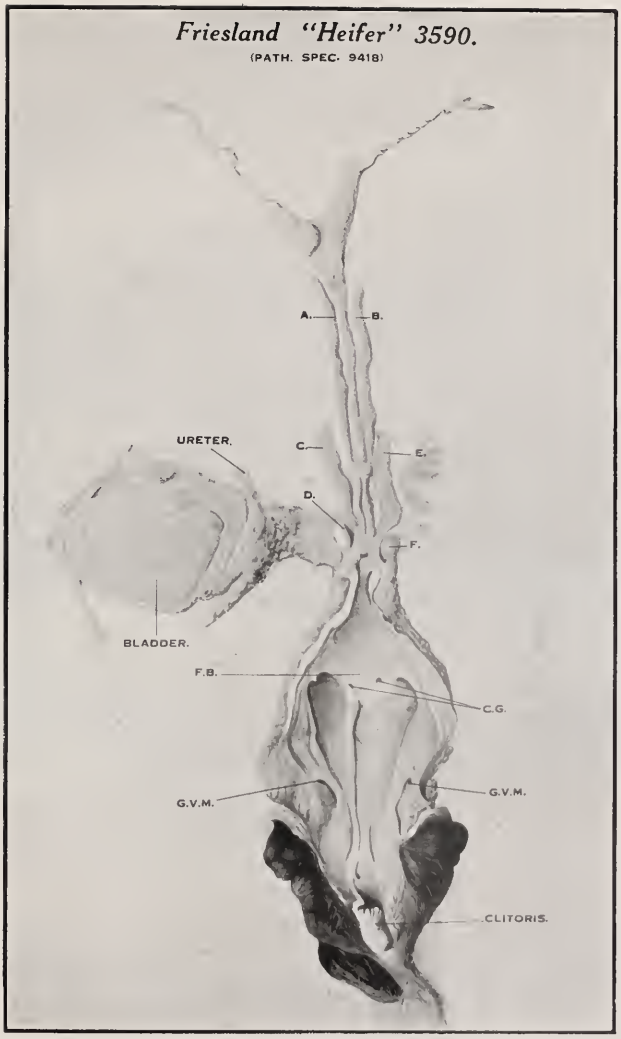
Friesland "Heifer" 3590.
(PATH. SPEC. 9418)
A.
B.
URETER.
C.
E.
D.
F.
BLADDER.
F.B.
C.G.
G.V.M.
G.V.M.
CLITORIS.

Fig. 2.

FIG. 3.

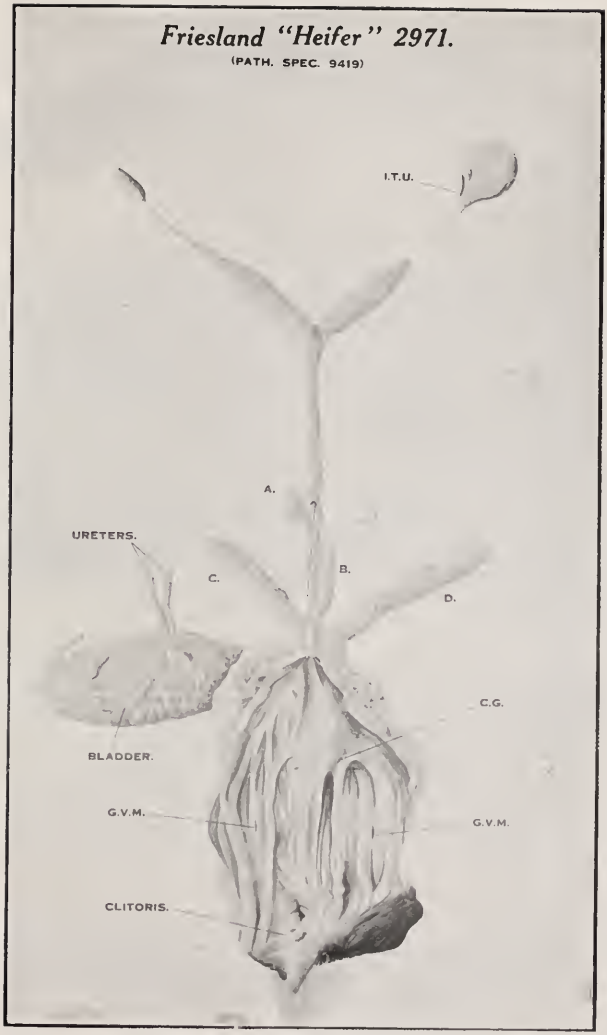
Friesland "Heifer" 2971.
(PATH. SPEC. 9419)
I.T.U.
A.
URETERS.
C.
B.
D.
C.G.
BLADDER.
G.V.M.
G.V.M.
CLITORIS.

FIG. 4.

FIG. 5.

Anatomical Studies, No. 17 : Epidermoid Cysts in Domesticated Animals.

By Professor H. H. CURSON, F.R.C.V.S., Dr. Med. Vet. and A. D. THOMAS, D. V. Sc., Veterinary Research Officers, Onderstepoort.

Introduction.

As epidermoid cysts are uncommon in domesticated animals, it is considered that the following note will prove of interest to veterinarians. According to Joest (1924) epidermoid cysts are particularly rare in the dog and pig.

(*a*) *Cyst from subcutis of shoulder* (*horse*).

This specimen (Path. No. 3,228, dated 14/11/23) actually consisted of 2 cysts, marked A and B in Fig. 1. The length of A was 11 cm. and, as will be noticed in the sketch, its free pole presented a constriction which divided the main cyst into two cavities, a smaller with a maximum diameter of 1.5 cm. and a larger 4.5 cm. in diameter. A narrow channel, 0.5 cm. in width existed between these cavities, each of which contained a firm, smooth, putty-like mass of material of sebaceous origin. The cyst marked B, approximately spherical and 4 cm. in diameter, was remarkable in that its contents were not only (*a*) sebaceous, but also consisted of (*b*) hair, the greater part of which was in a tangled mass and free from the wall. The hairs varied in colour from dark brown to white, and some attained a length of 5 cm. the majority, however, being less than this. Relatively few hairs were attached to the cyst wall. The sebaceous material occurred, not in a single mass as before, but as numerous small firm pellets (approximately 60 in number), and having a diameter of 0.5 cm. Some, however, were half this size. The wall of the cyst consisted of dense fibrous tissue varying in diameter from 1-2 mm., and, possessing a smooth non-pigmented inner surface. (See Fig. 2).

(*b*) *Cyst from subcutis of shoulder* (*ox*).

This structure (Path. No. 6,892 of 25/5/27) ovoid in shape (8.5 cm. × 5 cm.), contained a firm mass composed almost entirely of matted hair, kept together by sebaceous material and cast epithelium. On being disturbed in the preserving fluid, shreds or flakes separated from the main mass. The hairs were either black or white and the usual length varied from 4-5 cm. The cyst wall was strong and fibrous and had a thickness of approximately 2 mm. Characterising its inner surface, were diffuse patches of pigmentation and an abundance of adhering hairs, which lined the cavity in the same way that hairs are arranged in a bird's nest.

(c) Cyst from subcutis sternal region (sheep).

While conducting a post-mortem examination (12/9/29) on a full mouth merino wether (24,794), one of us (A.D.T.) encountered a circumscribed but fluctuating subcutaneous swelling in the right presternal region (Path. No. 9,617). Owing to the growth of wool, this was detected only upon removal of the skin. The cyst measured 6×5 cm. and on incision a small quantity of turbid fluid escaped. The contents of the swelling were white and composed of a fatty substance and matted wool. The wall of the cyst was thin (0.5 mm.) but strong, its inner surface being smooth and lined with fine wool.

General Histology.

As described by Delafield and Prudden (1928), the simplest forms of epidermoid cysts are lined by epithelium of the squamous type, whereas in the more complicated structures there are present hair follicles, and sweat and sebaceous glands.

Fig. 2 prepared from the horse, cyst B, shows the squamous nature of the epithelium, although hair follicles and sebaceous glands, undoubtedly present, are not observed in the section. Hair follicles, sebaceous glands and sweat glands are well demonstrated, however, in Fig. 3, prepared from the cyst of the ox. Fig. 4, representing a section of the cyst wall of the sheep, shows not only a hair follicle but also pronounced cornification of the superficial squamous layer.

General Embryology.

The origin of cysts such as described above, is not well understood. In all probability they arise as a result of displacement of the embryonic epidermis and are therefore congenital.

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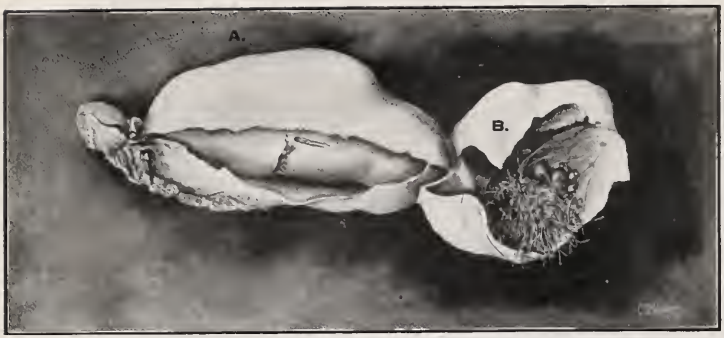

FIG. 1.

FIG. 2.

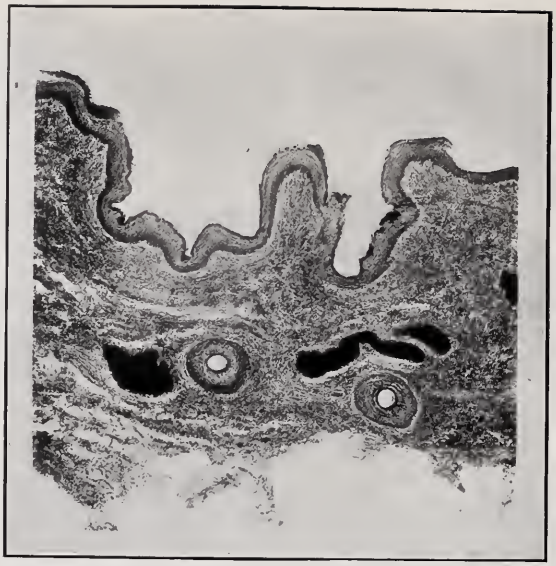

FIG. 3.

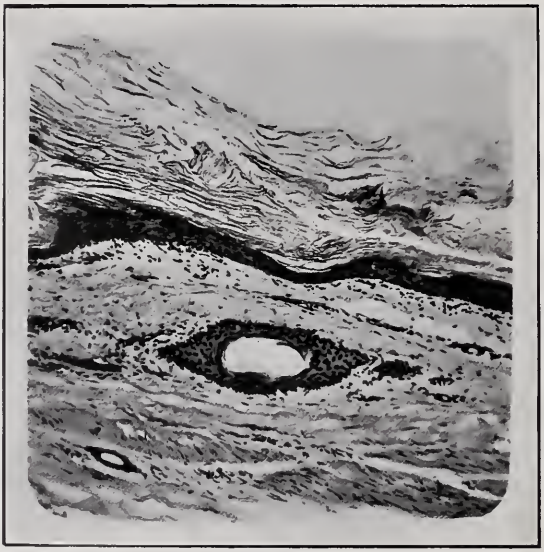

FIG. 4.

16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, August, 1930.

Anatomical Studies, No. 18 : Dental Anomalies in the Ox and Horse.

By Professor H. H. CURSON, F.R.C.V.S., Dr. Med. Vet., Veterinary Research Officer, Onderstepoort.

Dental anomalies of teratological origin are less common in the domesticated animals than in man. For this reason the following records are not without interest.

(*a*) Supernumerary Incisors in an Ox.

This specimen (6,514) was handed to Mr. R. B. Osrin, B.V.Sc. at the end of 1926, by a farmer in the Vryburg District with the history that "it came originally from Rhodesia" (File 144/474). Mr. Osrin thereupon kindly forwarded the specimen to this Institution.

There were visible a total of 29 permanent incisors which could be arranged, as shown in Figs. A and B, in 4 irregular rows of 8, 8, 9, and 4, i.e. estimating from within outwards. Taking the symphysis mandibulae as the middle line, neither the 2 halves of the corpus mandibulare nor the arrangement of the teeth was symmetrical. The teeth of the first or caudal row (See Fig. B. 1, 2, 3, etc.) were not only irregularly worn, but their situation was abnormal, the 3rd right incisor (7) being placed sideways. The incisors on the second row (See Fig. B, I, II, III etc.) were even more grotesque, the 1st right incisor (V) being fused with No. iii tooth of the third row. (See A, i, ii, iii, etc.). Tooth vi of this row is immediately cranial to vii and is shown in Fig. B. (See arrow.) The fourth row consisted of four irregular teeth that had not yet erupted.

The following specimens are taken from a collection of equine teeth presented to this Institution by the late T. B. Goodall, F.R.C.V.S., F.L.S. of Hampshire.

(*b*) Missing right corner Incisor.

This anomaly, occurring in the upper jaw of a 5 year old horse, is shown in Figs. C and D, making a detailed description unnecessary.

(*c*) Supernumerary Molar in a Horse.

This specimen (see Fig. E) shows not only the 1st premolar (wolf tooth), which is more common in the maxilla (as in the case in question), but also a 4th molar in the right upper jaw. The age of the subject at death was definitely over 4 years, and the wolf tooth had not been shed. This is, of course, not unusual. As will be seen in Fig. E the supernumerary molar had not yet reached the general wearing level of the remaining cheek teeth.

(*d*) Fusion of the 2nd and 3rd Deciduous Incisors.

In the case in question (see Fig. F) it was the teeth of the right upper jaw of the foal that were so involved. According to a note left by the donor and dated 10/5/25, the specimen was obtained at birth from the foal (horse). This anomaly is particularly rare.

Embryologically the conditions described above are of especial interest, representing as they do some defect of the dental lamina. In no case, however, was there mal-development of the enamel organ for in no instance (except *b*) was enamel wanting.

Fig. A.

Fig. B.

Fig. C.

Fig. D.

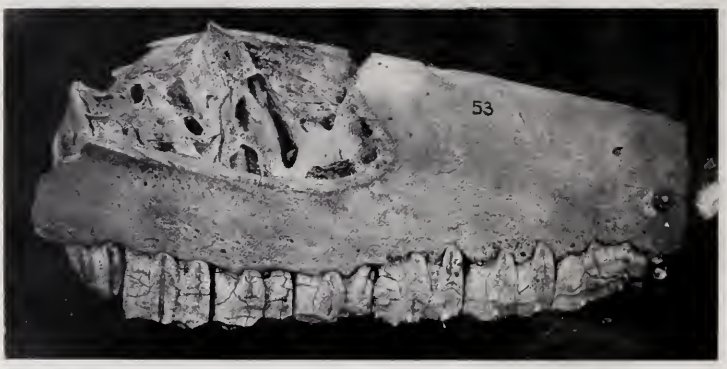

FIG. E.

FIG F.